

University of Alberta

**Effect of Preoperative Interventions on the Outcome Following Liver
Resection in a Rat Model of Cirrhosis**

by

Michael A. J. Moser ©

A thesis submitted to the Faculty of Graduate Studies and Research in
partial fulfillment of the requirements for the degree of Master of Science

in

Experimental Surgery

Department of Surgery

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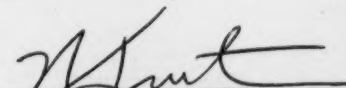
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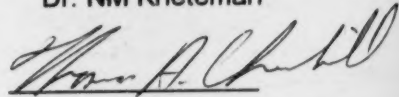
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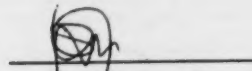
The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Effect of Preoperative Interventions on the Outcome Following Liver Resection in a Rat Model of Cirrhosis** by Michael A.J. Moser in partial fulfillment of the requirements for the degree of Master of Science in Experimental Surgery.



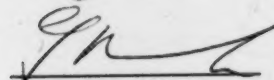
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ABSTRACT

Sixty-four CCl₄-induced cirrhotic rats were randomized to receive saline 1 cc / 200 g (untreated cirrhotic controls), ciprofloxacin 100 mg/kg, pentoxifylline 32 mg/kg, or a concentrated form of Traditional Chinese Herbal Remedy (TCHR), 1 cc / 200 g. An additional 12 (group 5) rats served as healthy, non-cirrhotic controls, and received no CCl₄, ethanol, or therapeutic interventions. Treatments were administered daily by gavage for four weeks, after which all rats underwent a 70% partial hepatectomy.

Total serum bilirubin was significantly improved in the ciprofloxacin (1.87 ± 0.25 mg/dL, $p < 0.05$) and pentoxifylline (1.40 ± 0.15 mg/dL, $p < 0.01$) treated groups, but unchanged in the TCHR group (2.21 ± 0.47 mg / dL), compared to untreated cirrhotic controls (3.00 ± 0.37 mg/dL). All five groups had similar postoperative serum AST levels. Regeneration, as assessed by ³[H]-Thymidine incorporation, was also significantly improved in the pentoxifylline treated group (17.8 ± 2.2 DPM / μ g DNA, $p = 0.05$) compared to untreated cirrhotic controls (9.9 ± 1.9 DPM / μ g DNA), but unchanged in the ciprofloxacin (16.1 ± 1.8 DPM / μ g DNA) and TCHR (10.9 ± 1.2 DPM / μ g DNA) groups. PCNA protein determinations were in keeping with ³[H]-Thymidine results. Fibrosis as measured by computer-assisted quantitation of collagen in the ciprofloxacin and pentoxifylline treated groups were significantly reduced (5.5 ± 0.6 % and 4.6 ± 0.2 %) ($p < 0.05$ and $p < 0.005$ respectively), compared to the untreated cirrhotic control group (8.1 ± 0.7 %), but unchanged in the TCHR group (6.6 ± 1.0 %). Procollagen type I mRNA expression was similar in the four cirrhotic groups.

In conclusion, of the interventions tested, pentoxifylline was the most useful in improving outcome following resection of cirrhotic liver in this model, and further studies with pentoxifylline are warranted.

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TABLE OF CONTENTS

FIGURES

TABLES

ABBREVIATIONS USED

ACKNOWLEDGEMENTS

ABSTRACT

THE CLINICAL PROBLEM : OVERVIEW	1
HEPATIC FIBROSIS	4
INTRODUCTION	4
THE SPACE OF DISSE	4
CELLULAR BASIS OF FIBROSIS	5
CYTOKINES INVOLVED IN FIBROSIS	6
COLLAGEN DEGRADATION: FIBRINOLYSIS	7
LIVER REGENERATION	8
INTRODUCTION	8
THE CELL CYCLE OF HEPATOCYTE PROLIFERATION	9
REGENERATIVE STIMULI	9
PROMOTERS AND INHIBITORS	10
MOLECULAR STEPS INVOLVED IN REGENERATION	10
ABNORMAL REGENERATION IN CIRRHOTIC LIVER	11
WHY IS REGENERATION POOR IN CIRRHOTIC LIVERS?	12

CIRRHOSIS	15
DEFINITION	15
EPIDEMIOLOGY	15
CLASSIFICATION	15
ETIOLOGY	16
OUTCOME AND PROGNOSIS	21
CURRENTLY AVAILABLE TREATMENTS	22
PREVIOUS TREATMENTS INVESTIGATED	22
HEPATIC RESECTION AND CIRRHOSIS	25
HEPATOCELLULAR CARCINOMA	29
INCIDENCE	29
ETIOLOGY	29
RELATIONSHIP BETWEEN CIRRHOSIS AND HCC	31
TREATMENTS	34
LIVER RESECTION <i>VERSUS</i> TRANSPLANTATION	40
THE CLINICAL PROBLEM	44
MORBIDITY AND MORTALITY	44
COMPLICATIONS	46
OTHER FACTORS	49
TREATMENTS EXAMINED IN THE STUDY	51
CIPROFLOXACIN	51
STRUCTURE	51
ABSORPTION	51
METABOLISM AND EXCRETION	52
DOSE	52
ACTIONS	53
PENTOXIFYLLINE	56
STRUCTURE	56
ABSORPTION	56
METABOLISM AND EXCRETION	57
MECHANISM OF ACTION	58
SIDE EFFECTS	59

TRADITIONAL CHINESE HERBAL REMEDY	61
OVERVIEW OF THE STUDY	63
HYPOTHESIS	63
STUDY DESIGN	63
MODEL OF CIRRHOSIS	63
MODEL OF RESECTION	65
TIMING OF OUTCOME ASSESSMENT	66
SURVIVAL	67
LIVER FUNCTION	67
ASSESSMENT OF REGENERATION	69
QUANTITATION OF FIBROSIS	72
METHODS	74
ANIMALS	74
CIRRHOSIS MODEL	74
TREATMENT GROUPS	74
SURGERY	77
LIVER FUNCTION DETERMINATIONS	80
HEPATIC REGENERATION ASSAYS	81
QUANTITATION OF FIBROSIS	84
STATISTICAL ANALYSIS	86
RESULTS	87
SURVIVAL	87
LIVER FUNCTION	93
HEPATIC REGENERATION	96
FIBROSIS	102
DISCUSSION	109
SUMMARY	109
CIPROFLOXACIN	110
PENTOXIFYLLINE	112
TRADITIONAL CHINESE HERBAL REMEDY	116
REGENERATION	117
OTHER POTENTIAL PROBLEMS	122
CLINICAL RELEVANCE	123

CONCLUSIONS	128
TOPICS FOR FUTURE STUDY	129
BIBLIOGRAPHY	130

TABLES

<i>Table I : Measures used in the assessment of outcome</i>	79
<i>Table II : Survival during the four-week treatment period</i>	92
<i>Table III : Fibrosis grade for the five groups</i>	105
<i>Table IV : Severe versus mild to moderate fibrosis</i>	106

FIGURES

<i>Figure I : The space of Disse in the fibrotic liver.</i>	4
<i>Figure II : Structure of ciprofloxacin</i>	51
<i>Figure III : Structure of pentoxifylline</i>	56
<i>Figure IV: Summary of the experimental protocol</i>	76
<i>Figure V: Equations for the calculation of restituted liver mass.</i>	81
<i>Figure VI : Rat survival curve with induction of cirrhosis.</i>	89
<i>Figure VII : Rat weights during the induction of cirrhosis.</i>	90
<i>Figure VIII : Serum AST 24 hours post partial hepatectomy</i>	94
<i>Figure IX : Serum bilirubin 24 hours post-partial hepatectomy</i>	95
<i>Figure X : Percent Restituted Liver Mass 24 hours post-partial hepatectomy</i>	97
<i>Figure XI : Thymidine Incorporation 24 hours post partial hepatectomy</i>	98
<i>Figure XII: Thymidine incorporation, including healthy controls.</i>	99
<i>Figure XIII : PCNA protein expression 24 hours post-partial hepatectomy</i>	101
<i>Figure XIV : Representative sections from each of the four cirrhotic groups</i>	103
<i>Figure XV : Fibrosis index</i>	104
<i>Figure XVI : Procollagen type I, alpha 1 chain mRNA expression.</i>	108
<i>Figure XVII : Thymidine incorporation as a function of percent resected liver mass</i>	121
<i>Figure XVIII : Diagram illustrating the effect of increasing regeneration in cirrhosis</i>	127

ABBREVIATIONS USED

ARDS	adult respiratory distress syndrome
AST	aspartate aminotransferase
CAH	chronic active hepatitis
cAMP	cyclic adenosine monophosphate
CC	cirrhotic controls
$\text{CCl}_3^{\bullet}$	trichloromethyl (free radical)
CCl_4	carbon tetrachloride
dCTP	deoxy-cytosine triphosphate
ddH ₂ O	double-distilled water
DABA	3,5-diaminobenzoic acid
DMNA	dimethyl nitrosamine
ECM	extracellular matrix
EDTA	ethylenediaminetetraacetic acid
GABA	γ -aminobutyric acid
HB _s Ag	hepatitis B surface antigen
HBV	hepatitis B virus
HCC	hepatocellular carcinoma
HCV	hepatitis C virus
HC	healthy controls
ICG	indocyanine green

MMP	matrix metalloproteinase
OD ₂₆₀	optical density for λ (wavelength) of 260 nm
ODC	ornithine decarboxylase
OTC	ornithine transcarbamylase
PBC	primary biliary cirrhosis
PCNA	proliferating cellular nuclear antigen
PDGF	platelet derived growth factor
PGE ₂	prostaglandin E ₂
PSC	primary sclerosing cholangitis
PT	prothrombin time
PTT	partial thromboplastin time
RES	reticulo-endothelial system
SDS	sodium dodecyl sulfate
SR	sustained release
SEM	standard error of the mean
TAE	trans-arterial embolisation
TCA	tricarboxylic acid
TCHR	traditional Chinese herbal remedy
TGF- α	transforming growth factor - alpha
TGF- β	transforming growth factor - beta
TIMP	tissue inhibitor of metalloproteinase
TK	thymidine kinase

TNF

tumor necrosis factor

$t_{1/2}$

half life

THE CLINICAL PROBLEM : OVERVIEW

The need for hepatic resection in the setting of cirrhosis makes for a dangerous combination; unfortunately, this is not an uncommon clinical situation. The most common indication worldwide for hepatic resection is hepatocellular carcinoma (HCC) which constitutes approximately 90% of all primary malignant liver tumors. Moreover, 50-85% of patients with HCC have associated cirrhosis [1]. Conversely, 10 - 20 % of patients with cirrhosis from any etiology will go on to develop HCC [2,3].

In a patient with a normal liver, resections of up to 75% or more of liver mass can be achieved with relative safety and mortality rates of 5% or less are possible [4]. Such 'massive' resections are possible in large part because of the tremendous ability of normal liver to regenerate its lost mass and function.

Resection of cirrhotic liver, on the other hand, presents a very difficult clinical situation. Mortality rates of 10 - 50% and complication rates of 50 - 100 % are commonly quoted, and this despite careful preoperative workup and rejection of large numbers of patients who are medically unfit [2,5]. There are a number of factors which make it difficult for the cirrhotic patient to tolerate resection, including poor baseline liver function, impaired regeneration, and portal hypertension. The resection of tissue from a liver with already impaired function is fraught with the risk of hepatic decompensation and death, as a minimum amount of liver function appears to be necessary to sustain life.

Unlike the normal liver, the cirrhotic liver has severely impaired regenerative activity. Until recently, the cirrhotic liver was thought to be unable to regenerate at all [6]. However, we now know that while regeneration is possible in the cirrhotic liver, the process is greatly impaired both in rate and extent [7]. The reason for this impaired regenerative capacity is currently the subject of numerous investigations.

A number of treatments have been proposed to improve liver function and fibrosis in the cirrhotic liver [8]. However, the effects of these interventions and whether they could be helpful in the setting of the cirrhotic liver undergoing partial hepatectomy remain to be determined. Improvements in hepatic function and regenerative ability could make the cirrhotic liver more tolerant to resection and lead to an increase in the number of surgical cures for hepatic malignancies.

The purpose of this study was to document the effect of three preoperative interventions that have been reported to be effective in the treatment of cirrhosis, on the outcome after partial hepatectomy in a rat model of cirrhosis.

The hypothesis is that the three preoperative interventions (ciprofloxacin, pentoxifylline and Traditional Chinese Herbal remedy (TCHR)) will be useful in improving the outcome of hepatic resection in terms of function, regeneration, and fibrosis in this cirrhotic rat model.

In this introduction, we first review the basic processes of fibrosis and regeneration, both of which are involved in the pathology of cirrhosis. As mentioned above, a common situation where there is a need for hepatic

resection of cirrhotic liver is hepatocellular carcinoma, and this neoplasm will be reviewed extensively, along with a discussion of treatments currently available. Finally, the specific clinical question which is to be addressed, namely hepatic resection in the setting of cirrhosis, will be discussed in depth, and will serve to introduce the treatments which will be tested in this study.

HEPATIC FIBROSIS

INTRODUCTION

Hepatic fibrosis can be defined as an increase in extracellular matrix (ECM) components, particularly collagen types I and III, in response to hepatic injury [51]. The process involves two components: proliferation of fibroblasts and increased deposition of collagen [9], and can be likened to the formation of scar tissue in wound healing. Collagen deposition is the net result of fibrogenesis (collagen production), and fibrinolysis (collagen degradation).

THE SPACE OF DISSE

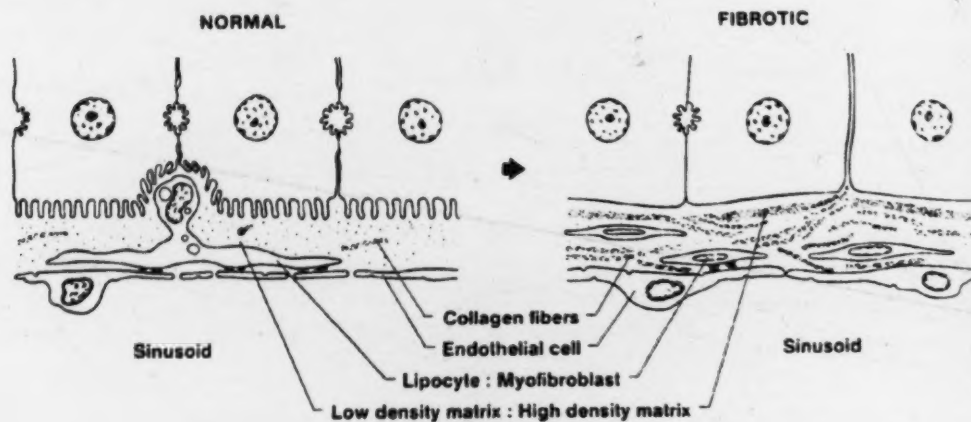


Figure 1 : The space of Disse in the fibrotic liver (from Bissell DM. Cell matrix interaction and hepatic fibrosis. *Progr Liver Dis* 1990;9:143-55).

Deposition of ECM is diffuse, however changes to the "space of Disse" appear to be of particular importance [10]. The space of Disse lies between the endothelium and the hepatocyte sinusoidal membrane and normally contains a loose matrix of connective tissue. In chronically injured liver, the space of Disse becomes filled with a dense matrix of collagen and fibronectin [11]. The result is the loss of endothelial cell pores, a process termed 'capillarisation' [12], and marked impediment of the exchange of molecules between the sinusoidal space and hepatocytes [13]. It has been proposed that collagen deposition in the space of Disse is responsible for the abnormal function and regeneration seen in liver diseases [14].

CELLULAR BASIS OF FIBROSIS

Many cell types and signaling molecules are thought to be involved in the process of fibrosis. Central to the process is the Ito cell which resides in the space of Disse, and which is also known by a number of other names, including lipocyte, hepatic stellate cell, pericyte, and fat-storing cell [15]. Following liver injury, the Ito cell becomes activated. The mechanism whereby liver injury stimulates Ito cell activation is thought to involve hepatic macrophages ("Kupffer cells") and platelets [16], based on *in vitro* studies. Proliferation of Ito cells and the secretion of collagen by Ito cells and fibroblasts [14] is influenced by the cytokines transforming growth factor- beta (TGF- β) and platelet-derived growth factor (PDGF), two of the most important cytokines involved in the production of fibrosis identified to date.

Despite some studies suggesting a link between Ito cells and myofibroblasts, the source of the latter cell line remains controversial. Ito cells in culture can be observed to undergo changes in their appearance that render them similar to that of myofibroblasts such as the expression of smooth muscle α -actin and production of certain cell surface receptors [17]. However, in the *in vitro* model at least, Ito cells differ from myofibroblasts with respect to the presence of desmin, a cell-membrane glycoprotein, in the latter [13].

CYTOKINES INVOLVED IN FIBROSIS

TGF- β is produced by Kupffer cells, platelets, and Ito cells themselves, implying at least a partial autocrine mechanism [15]. Its main actions are to activate Ito cells and promote collagen production [18]. A further, well documented action is the inhibition of DNA synthesis which may be important in controlling excessive regeneration [19].

PDGF was initially isolated from platelets, however subsequent analyses have shown that it is also produced by Kupffer cells and endothelial cells in the liver [20]. The main role of PDGF appears to be Ito cell activation and Ito and fibroblast cellular proliferation [21]. This role has been studied in serum samples obtained from patients with liver disease [22].

COLLAGEN DEGRADATION: FIBRINOLYSIS

Another important aspect of fibrosis is fibrinolysis or degradation of extracellular matrix components. The mechanisms and control of metalloproteinases, a group of enzymes involved in fibrinolysis, are just beginning to be explored. Matrix metalloproteinases (MMP) appear to be synthesized primarily by Kupffer cells [23]. Decreased MMP activity has been suggested as a mechanism of excessive collagen deposition in animal models of fibrosis [23].

A specific subgroup of MMP's, the collagenases, also seem to be involved in ECM remodeling that occurs to accommodate the cell proliferation that is seen in regeneration [24]. Tissue inhibitors of matrix metalloproteinases (TIMP's) have been identified, and increased levels have been found in the serum of alcoholic patients with cirrhosis [25].

Overall, fibrosis is the net result of fibrogenesis and fibrinolysis. Fibrogenesis involves Ito cell activation and proliferation, which is mediated by cytokines such as TGF- β and PDGF, with the end result being collagen deposition by myofibroblasts. Collagen deposition in the space of Disse may be responsible for the abnormal function and regeneration seen in cirrhotic livers. Each step involved in ECM production and deposition presents a potential target in the treatment of fibrosis and cirrhosis [8,26].

LIVER REGENERATION

INTRODUCTION

The liver is unique in that it is the only organ which is capable of extensive regeneration in humans. Regeneration can be defined as an increase in cell size and cell number, usually following injury or resection, such that the original mass and function is restored [27].

No discussion of liver regeneration would be complete without mentioning the first account of regeneration, which is found in Greek mythology. Prometheus was punished by Zeus for stealing fire from mount Olympus and introducing it to the mortals. He was tied to a rock and had his liver fed to an eagle. Overnight, his liver would grow back and the next day the eagle would return to feed again over and over [28].

The ability of the healthy liver to regenerate can be dramatic. One of the first demonstrations of regeneration was in 1931 in a classic study by Higgins and Andersson [29]. Following 68% resection in the white rat, the remaining lobes regenerated to attain the initial liver mass prior to resection within less than 4 weeks. In humans, the process is less rapid, but just as complete [30]. Regeneration is illustrated by the transplantation of small-for-size or living-related liver grafts, which grow to attain the appropriate mass for body size [31].

THE CELL CYCLE OF HEPATOCYTE PROLIFERATION

The cell cycle of mitosis, begins with the G1 phase and is followed by the S (DNA synthesis) phase, the G2 phase, and the M phase, during which cell division occurs. In the normal, quiescent, nonregenerating liver, mitosis is seen in very few hepatocytes (1 in 100 000) [30]. Almost immediately following an appropriate regenerative stimulus, regeneration is initiated. Changes in hepatocyte membrane potentials [32] and transcription of immediate-early protooncogenes [33] have been documented within minutes of partial hepatectomy.

A central event in hepatic regeneration is DNA synthesis which occurs in the S phase. Indeed, most of the methods used to measure regeneration depend to some extent on an index of DNA synthesis which is occurring in hepatocytes.

REGENERATIVE STIMULI

A number of stimuli have been identified in the initiation of regeneration; these can be broadly grouped as tissue loss (from resection), or pathologic damage to multiple hepatocytes (viral, toxic, or autoimmune injury) [27]. The precise regulation of regeneration in response to these stimuli is controlled by a complex array of promoting and inhibiting factors. Such a homeostatic mechanism is necessary to guard against uncontrolled and excessive liver growth.

PROMOTERS AND INHIBITORS

A large number of these regulatory substances are of extrahepatic origin and are delivered via the portal vein to the liver [34]. Over fifty such promoters (insulin, glucagon, and amino acids) and inhibitors (γ -aminobutyric acid (GABA), octreotide, mercaptans, and ammonia) have been identified. In addition, intrahepatic substances have been identified which are produced in hepatocytes or Ito cells. Examples of these include hepatocyte growth factor (HGF) [35], transforming growth factor- α (TGF- α) [36], epithelial growth factor (EGF)[36], and TGF- β [19].

MOLECULAR STEPS INVOLVED IN REGENERATION

While many substances have long been known to be promoters or inhibitors of regeneration, the molecular steps involved in hepatocytes entering the cell cycle (G0- G1) are just now beginning to be elucidated with the advent of newer molecular techniques.

Among the earliest changes seen are membrane depolarisation and transcription of immediate-early protooncogenes c-fos, c-jun, and c-myc. Membrane depolarisation occurs **immediately** and persists throughout the period of active DNA synthesis (~18 hours) before returning to baseline [27]. The mechanism whereby membrane depolarization occurs is unclear but the process can be prevented by pre-operative administration of GABA which has also been reported to inhibit hepatic regeneration [37]. Transcription of the protooncogenes occurs **within minutes** in a specific sequential fashion [38].

The protein products of c-fos and c-jun combine to form the AP-1 transcriptional activating factor [38]. Delayed early genes, such as p53, are expressed within 4 to 8 hours [38]. Finally, at 16 to 24 hours, activation of the ras family is seen [39]. DNA synthesis, a central event in regeneration begins around 8-12 hours after the regenerative stimulus and peaks around 24 hours, after which a lower "plateau" of DNA synthesis is maintained for several days or weeks [27].

ABNORMAL REGENERATION IN CIRRHOTIC LIVER

In a patient with a normal liver, it is possible to resect 70-80% of liver mass with low morbidity and mortality rates. Healthy liver possesses tremendous regenerative ability and can regenerate lost mass and function even following resections of 80% or more. On the other hand, cirrhotic liver has long been known to possess severely impaired regenerative activity. Indeed, for many years, the cirrhotic liver was thought to be unable to regenerate at all [6].

Using serial CT scans, Nagasue et al. examined 10 cirrhotic patients who had undergone hepatic resection [7]. The group was able to document regeneration in these cirrhotic livers, although the rate and extent of the regeneration was slower than normal.

In a CCl₄ induced cirrhotic rat model, one group showed that regeneration was occurring in cirrhotic rats following 70% hepatectomy as evidenced by significantly higher ³[H]-Thymidine incorporation values and PCNA labeling indices, compared to those undergoing sham operations. However, regenerative indices were markedly lower in the cirrhotic rats compared to

concurrently studied noncirrhotic rats following partial hepatectomy [40]. No precise values were listed in the paper, only charts. The PCNA labeling index at 24 hours was in the order of 20% for the cirrhotic rats and 50% for the control rats [40]. ^3H -Thymidine incorporation was of the order of about 1200 DPM / μg DNA for control rats and only about 200 DPM / μg DNA for the cirrhotic rats [40].

WHY IS REGENERATION POOR IN CIRRHOTIC LIVERS?

The reason for the impairment of regeneration in cirrhotic liver is unclear. A number of hypotheses have been proposed, including a deficiency of growth promoters, an excess of growth inhibitors, abnormal cell-to-cell communication, and abnormal hepatocyte membrane signaling mechanisms [41]. Although it is likely that each of these factors is involved to a certain extent, the loss of growth promoters and presence of circulating inhibitors have been the most extensively studied areas to date [42].

As stated previously, the regenerative response is under the control of a complex network of promoters and inhibitors, and one possibility is that there is a paucity of promoters responsible for the poor regeneration seen with cirrhosis. The fact that levels of HGF and TGF- α have been documented and found to be elevated rather than decreased in fulminant hepatic failure and cirrhosis [43] renders this possibility less likely.

Current research favors the possibility that an excess of inhibitors interferes with regeneration. Two animal studies [42,44] examined the effects of three toxins known to accumulate in fulminant hepatic failure (a condition associated with impaired hepatic regenerative activity), namely, ammonia, octanoic acid, and mercaptans. In these studies, rats subjected to 70% partial hepatectomies were given 1-4 doses of one of the toxins over the 24 hour period following surgery. All rats receiving the toxins had markedly suppressed

regenerative indices, which included ^3H -Thymidine incorporation, and thymidine kinase (TK) and ornithine decarboxylase (ODC) activity assays.

GABA, an amino acid neurotransmitter, is a potent inhibitor of hepatic regeneration [45]. GABA is largely metabolized in the liver by GABA transaminase and liver dysfunction has been associated with elevated serum GABA [45]. Elevated GABA serum levels may be involved in the pathogenesis of hepatic encephalopathy [46].

As mentioned above, the deposition of fibrotic tissue in the space of Disse leads to capillarisation of endothelial cell pores and interferes with the exchange of molecules and synthesized products between the hepatocytes and the sinusoids. Thus, cell to cell communication is abnormal [14]. Given the complex network of cells and cytokines involved in normal regeneration, it is hypothesized that collagen deposition in the space of Disse is at least partially responsible for the abnormal regeneration seen in cirrhotic livers [14].

CIRRHOSIS

DEFINITION

Cirrhosis is a chronic liver disease which occurs in response to various viral, chemical, and autoimmune insults. A definition of cirrhosis includes four components: cell death in response to chronic insults, deposition of excessive connective tissue, attempted regeneration by surviving cells, and architectural distortion [47]. The vascular disorganization which occurs is the basis of many of the serious complications of cirrhosis, which include portal-systemic shunting and encephalopathy, variceal hemorrhage and portal hypertensive gastropathy [48].

EPIDEMIOLOGY

Cirrhosis is one of the ten leading causes of death world wide, particularly in males. In Canada in 1989, cirrhosis was the seventh leading cause of death, with 2238 deaths due to cirrhosis [49]. A prevalence of 15.0 per 100 000 has been reported in North America [48]. The costs associated with treating the complications of cirrhosis are enormous, particularly when dealing with variceal bleeding [50]

CLASSIFICATION

A number of classifications have been proposed for cirrhosis, including morphological, histological, and etiological.

The morphological classification divides liver disease into micronodular and macronodular cirrhosis. This classification is useful despite its simplicity, as it often provides clues to etiology, and is more consistent than when histological or etiological criteria are applied [51].

Micronodular cirrhosis is said to be present when the majority of nodules are less than 3 mm in diameter. Micronodular cirrhosis is seen in alcoholic cirrhosis, hemochromatosis, and nutritional cirrhosis [51].

Macronodular cirrhosis is characterized by coarse nodules, most of which are greater than 3 mm in size. Two subtypes of macronodular cirrhosis exist: the postnecrotic and posthepatitic types. In the postnecrotic type, seen following chronic viral hepatitis, broad fibrous bands separate large lobules and a very coarse appearance is seen. In the posthepatitic type, the fibrous bands are much more narrow and the suggestion has been made that this type follows a single episode of necrosis rather than repeated or ongoing insults [47].

A histological classification of cirrhosis includes portal, postnecrotic, posthepatitic, biliary, and venoocclusive cirrhosis.

ETIOLOGY

Many etiologies have been identified for cirrhosis, including infectious, toxic, cholestatic, autoimmune, and vascular causes.

Infectious

Most of the infectious causes of cirrhosis are viral in origin. Hepatitis B infection is one of the leading causes of cirrhosis in oriental countries [15].

There is progression from acute to chronic hepatitis in 5% of adults and 95% of newborns infected with the virus [47]. If the chronic hepatitis is active, chronic active hepatitis (CAH), the risk of developing cirrhosis is approximately 70% [15].

Hepatitis C, formerly referred to as Non-A, Non-B hepatitis, has taken on an increasing importance since the HCV virus was characterized in 1989. HCV has a particular propensity for becoming chronic (approximately 80% of cases regardless of age) and progressing to cirrhosis [15], however the true rates remain to be defined, as reliable tests for detecting the virus have only recently become available, and natural history studies are presently underway.

HCV as a cause of end-stage liver disease becomes highly significant when one considers the high number of Canadians infected with the virus. In the wake of the investigation into the Red Cross blood scandal, thousands of people have been screened in the last year. It is estimated that over 12 000 people became infected with HCV from tainted blood transfusions between 1986 and 1992 [52]. Four times that number of cases have been acquired through illicit intravenous drug use during that time [53]. Until more effective antiviral therapies are available, many of these patients will progress to cirrhosis and require liver transplantation. Thus, HCV cirrhosis is rapidly becoming the leading indication for liver transplantation [54], and this, coupled with the high rate of HCV reinfection post-transplant [55] places severe stresses on an already limited donor pool.

Toxic

Toxic damage to the liver may be caused as a result of direct and indirect actions. Important indirect acting agents include ethanol, CCl_4 , and acetaminophen. The toxicity of indirect-acting agents depends upon their metabolism by the cytochrome P450 pathway to form toxic intermediates. These toxic metabolites then act directly on various cell components leading to cellular damage [15].

Alcoholic cirrhosis is a leading cause of cirrhosis worldwide, particularly in western countries. Ethanol has both direct and indirect actions. Its primary hepatotoxic effect is indirect, with acetaldehyde as the principal toxic metabolite. Acetaldehyde causes cell membrane damage by binding to sulfhydryl groups and amino groups forming acetaldehyde-protein adducts that interfere with cell function [48]. Direct effects include mitochondrial damage and altered protein synthesis [48].

Cholestatic

Secondary biliary cirrhosis can result when there is chronic obstruction of the intrahepatic biliary tree as a result of stones, strictures, tumors (benign or malignant), or flukes [47].

Primary cholestatic disorders which can lead to biliary cirrhosis include primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). The precise etiology of these disorders is not known, but an autoimmune mechanism may be involved [56].

PBC is a chronic cholestatic disease characterized by destruction of small (interlobular and septal) bile ducts. The course is generally one of gradual deterioration and death unless liver transplantation is performed [57]. To date, no medical therapy has been shown to be effective in prolonging survival or time to transplant, although some symptomatic improvement has been observed with the use of ursodeoxycholic acid and methotrexate [58].

PSC involves larger intra- and extra-hepatic ducts, and stenosis is often worst at the bifurcation of the right and left hepatic ducts. Clinically, the course is one of recurrent bouts of cholangitis and progressive biliary cirrhosis [59]. The disease occasionally responds to medical treatments such as prednisone, ursodeoxycholic acid, and methotrexate [54], suggesting an autoimmune etiology. It is likely that some overlap exists between autoimmune chronic active hepatitis and the cholestatic diseases. Medical management is effective in lowering serum bilirubin and improving symptoms of pruritis, but its role in altering the natural history of the disease remains unproven [54]. Patients with predominantly extrahepatic disease may benefit from excision of the extrahepatic ducts, but this makes later liver transplantation more difficult. For this reason, interest has increased in percutaneous and endoscopic techniques for dilation or stent placement. In recent years, there has been a trend towards earlier transplantation in these patients, before other surgical interventions, and before cholangiocarcinoma develops [60].

Autoimmune

Idiopathic autoimmune chronic active hepatitis remains a constellation of poorly defined liver diseases, characterized by the presence of one of several autoantibodies, polyclonal hyperglobulinemia, and a favorable response to immunosuppression. A precise definition is still being sought, and in many instances, the diagnosis is one of exclusion [61]. Steroids or azathioprine have been shown to be effective in controlling the disease process to some extent, but many still require liver transplantation after many years of disease which has progressed to cirrhosis [61].

Vascular

Vascular causes of cirrhosis include cardiac cirrhosis, Budd-Chiari syndrome, and venoocclusive disease of the liver [15]. Cardiac cirrhosis results from long-standing right-sided heart failure or pericarditis. The condition is rarely seen clinically and is often only diagnosed on postmortem examination [15]. An equally uncommon condition is the Budd-Chiari syndrome which results from hepatic vein obstruction from thrombus or a membranous web. Obstruction to venous outflow leads to centrilobular congestion and hemorrhage which are easily identified histologically. Venocclusive disease of the liver consists of progressive narrowing and fibrosis of the terminal hepatic veins. Although originally described in individuals exposed to certain alkaloids found in bush teas, it is now more often seen in bone marrow recipients who have received combination chemo- and radiation therapy [48].

OUTCOME AND PROGNOSIS

Many studies have attempted to quantify the natural history and prognostic factors for survival in cirrhosis. Not surprisingly, the results reported to date vary widely with the group of patients studied, the cause of their liver disease, and the geographical location [62].

One particular study distinguished between patients with compensated versus uncompensated cirrhosis. Compensated patients had a ten-year survival rate of 47%, while decompensated patients, characterized by the presence of ascites, jaundice, gastrointestinal bleeding or encephalopathy, had a considerably worse survival of 16% at five years [63].

In alcoholic cirrhosis, with abstinence, the prognosis appears improved in some studies [64], while in other studies, no difference is seen whether the patient stops drinking or not [65]. Once again, the differences may be related to whether the cirrhosis is compensated or decompensated.

CURRENTLY AVAILABLE TREATMENTS

While many potential therapies have been studied as a means of treating cirrhosis, few (if any) have become accepted clinically. As a result, treatment of patients with cirrhosis is usually supportive, by managing the complications as they arise, particularly variceal bleeding, ascites, and encephalopathy.

In certain cases, specific therapies are available, but only for the treatment of the underlying etiology, and not the cirrhosis. Examples include the use of corticosteroid for autoimmune disorders, bile acid replacement therapy for cholestasis, iron depletion treatment for hemochromatosis, and penicillamine for Wilson's disease [48]. An important advance in the treatment of liver disease has been the development of the HBV vaccine to prevent infection. Once infection is established, however, interferon-alpha may be helpful in these patients as well as those with HCV infections [66].

PREVIOUS TREATMENTS INVESTIGATED

In the search for effective treatments of cirrhosis *per se*, a number of groups have focused on decreasing the amount of fibrosis. Many drugs have been studied; among the most notable are colchicine, prostaglandin E₂ (PG E₂), ciprofloxacin, and pentoxifylline, which will be discussed below.

Colchicine has been studied for many years and its effects on the cirrhotic liver have been well documented. In animal studies, colchicine decreases collagen production and secretion, and increases collagenase production [67].

Studies using the CCl₄ model of cirrhosis in rats have documented that colchicine decreased fibrogenesis and improved liver function [68]. In addition, colchicine has been studied in humans in a randomized-controlled trial which followed 100 patients for 3-14 years [69]. The study reported improved patient survival and liver histology in the group receiving colchicine versus the group receiving placebo. Unfortunately, drop out rates were high and the treatment and placebo groups were not well matched, making it difficult to interpret the results. In addition, comparisons were made based on subjective histological criteria, while no baseline histological data was available for comparison. Despite the early promising results in *in vitro* and animal studies, a number of more recent clinical trials have described conflicting results, and as a result, interest in this drug has diminished [70]. One study using an *in vitro* model of fibrogenesis noted that colchicine was only able to inhibit fibroproliferation at toxic serum levels [71]. Nonetheless, the drug is relatively non-toxic, and many physicians currently choose to treat cirrhotic patients with colchicine until a better alternative is found.

Prostaglandins are potent, short lived molecules whose effects have been documented in the kidneys, lungs, uterus, and many other organs [72]. Prostaglandins are involved in inflammation and hemostasis and induce smooth muscle contraction [72]. One prostaglandin, PG E₂ has been studied in liver regeneration after it was observed that PG E₂ increased following partial hepatectomy [73]. Meanwhile, Indomethacin, a known inhibitor of PG E₂

production, was shown to prevent increases in PG E₂ as well as DNA synthesis in the regenerating liver [73]. Years later, PG E₂ was shown to increase DNA synthesis in cultured hepatocytes [74]. The mechanism whereby prostaglandins promote regeneration is only recently beginning to be elucidated. Preliminary data suggest that PG E₂ enhancement of regeneration is a receptor-mediated process which is associated with intracellular increases in cAMP [75]

While PG E₂ has interesting theoretical actions, its extremely short half-life, as well as the fact that it must be given parenterally render its use impractical for chronic disorders such as cirrhosis. Furthermore, human trials undertaken to determine if PG E₂ could be useful in improving the outcome of patients with cirrhosis have not shown promising results. A small clinical trial examining the use of a synthetic derivative of PG E₂ in alcoholic cirrhosis documented only minimal changes in outcome, along with a conflicting result that the aminoterminal peptide of procollagen type III levels were unchanged [76].

Ciprofloxacin [77], pentoxifylline [20], and traditional Chinese herbal remedies [77] have been examined in animal models of cirrhosis and the mechanisms whereby they decrease fibrosis are beginning to be elucidated. These drugs will be discussed in greater detail later in this thesis.

HEPATIC RESECTION AND CIRRHOSIS

A thorough review of the literature reveals only a few studies directly examining the outcome following partial hepatectomy in cirrhotic liver. It is known that a liver with advanced fibrotic and cirrhotic changes possesses poor regenerative capacity [78,79], however the precise relationship between function, fibrosis, and regeneration has yet to be determined. As discussed earlier, many investigators have focused on improving hepatic function and the fibrotic state of the liver. However, only a few have focused on improving the regenerative response following partial hepatectomy to provide sufficient functioning liver mass to avoid hepatic decompensation and death.

Putrescine is a polyamine involved in promoting hepatic regeneration as well as the growth and development of many other tissues [80]. Indeed, putrescine and other polyamines, including spermidine, may be essential for regeneration to occur [81]. Putrescine has been shown to enhance regeneration in an animal model of acute liver disease [82]. One study examined the effect of putrescine at doses of 1, 10, and 100 mg / kg given before and after partial hepatectomy in a rat model of cirrhosis [83]. No effect on regeneration was observed as measured by ^3H -Thymidine incorporation and ornithine decarboxylase (ODC) activity. The lack of response could in part be due to the fact that only minimal increases in hepatocyte putrescine levels could be attained with exogenous therapy.

Transforming Growth Factor alpha (TGF- α) is one of several cytokines involved in the promotion of hepatic regeneration [84]. Kokudo and his group used the CCl₄ - induced rat model of cirrhosis to study the effect of TGF- α given after partial hepatectomy [78]. It was observed that cirrhotic rats treated with TGF- α achieved hepatic regeneration, as measured by ³[H]-Thymidine incorporation, comparable to that in non-cirrhotic rats. The authors conclude that the cirrhotic liver possesses a "relative" deficiency of TGF- α . The mechanism by which this deficiency occurs is unknown, but could be due to decreased production of TGF- α or impaired TGF- α delivery and uptake due to disturbed ECM architecture and abnormal cell-to-cell communication.

While TGF- α has been studied extensively as a cytokine involved in hepatic regeneration, further studies of TGF- α in a therapeutic role have not been done. There are many reasons for this: Firstly, the agent has a short half life ($t_{1/2}$ ~ 3 min) and an autocrine action [36] and would thus need to be given by continuous intravenous infusion. This may render the drug impractical in a clinical setting. Secondly, at this time, the cytokine is extremely expensive¹, and most of the studies have been limited to cell cultures, where small doses can be used. At this time, animal studies or clinical trials would have prohibitive costs. Thirdly, because TGF- α has been associated with tumor initiation and promotion [85], systemic administration of such an agent should probably be

avoided in the setting of premalignant tissues (cirrhosis) and in patients with malignancies or micrometastases.

An important study by Ito and associates suggested that while regeneration is important to ensure survival after partial hepatectomy, excessive regeneration may actually contribute to an impairment of liver function [86]. The group examined the relative expression of "housekeeping" (liver function) genes and protooncogenes (involved in liver regeneration) and found that the housekeeping genes, which included albumin and ornithine transcarbamylase (OTC), were significantly depressed at 24 hours post-hepatectomy, at a time when accelerated DNA synthesis was occurring. Simultaneously, increased transcription of protooncogenes c-fos, c-myc, and p 53 was occurring. The group speculated that regenerating hepatocytes enter a dedifferentiated state where they are unable to participate simultaneously in liver metabolic functions. Further studies examining the dedifferentiation of hepatocytes and the modulation of this response to hepatic resection are needed.

Thus, excessive regeneration may actually contribute to liver failure after hepatic resection of cirrhotic liver. Preoperative rather than postoperative interventions may be more important in improving the outcome of liver resections in this setting. Hence, this study focused primarily on the use of interventions administered preoperatively, rather than intra- or post-operatively. Certainly,

¹ We looked into the possibility of using this agent. Four weeks of treatment for 20 rats would have cost in excess of \$30 000, whereas the total cost of the pentoxifylline used in this study was about \$30!

interventions found to be useful preoperatively could also be useful if continued perioperatively.

HEPATOCELLULAR CARCINOMA

INCIDENCE

Worldwide, HCC is the most common solid tumor in males [15]. The incidence of HCC varies greatly from one geographic region to another, likely as a result of different prevalence of causative factors by region. For example, the age-adjusted incidence rate in Singapore in 1987 was 31.6 per 100 000 people, whereas in the United States, the rate was 4 per 100 000 [87]. In South Africa and Asia, HCC constitutes almost 40% of all cancers whereas in North America, HCC represents 2-3 % of all cancers [88].

ETIOLOGY

Many of the suspected etiologic agents for HCC have also been implicated in causing cirrhosis. Up to 85% of patients with HCC have coexisting cirrhosis [1], while conversely, 10- 20 % of patients with cirrhosis from any cause will develop HCC [2,3]. While cirrhosis appears to be a risk factor for HCC, the precise relationship remains to be fully elucidated.

The primary risk factors for HCC identified to date are HBV and HCV, as well as cirrhosis itself.

HBV

HBV is a leading cause of both cirrhosis and HCC and can be identified in the majority of cases of HCC in eastern countries [89]. Chronic carriers of HBV are at particular risk of developing HCC. The mechanism whereby HBV chronic infection leads to HCC is unclear, but may be related to the fact that HBV DNA integrates into host genomic DNA [90]. The relative risk of HCC developing in HBV chronic carriers has been reported as 30 to 160 times in various series [91,92].

HCV

The Hepatitis C virus was only identified in 1989 and clinical tests were made available for its detection shortly thereafter [93]. As a result, a large proportion of patients with HCC who were HBsAg negative were subsequently found to be anti HCV positive [90]. The mechanism of carcinogenesis is unknown; HCV, an RNA virus, does not integrate into the host genome and lacks reverse transcriptase [48]. It has been suggested that the carcinogenicity of HCV may be solely due to the induction of cirrhosis, which often follows chronic infection [15].

Chemical Carcinogens

Many chemical carcinogens have been identified in the causation of HCC, including Azo dyes, chlorinated hydrocarbons, and Aflatoxin B [15]. These appear to interfere with the tumor suppressor p53 gene or its protein product.

Aflatoxin B deserves special mention because its carcinogenic activity has been documented in epidemiological, *in vitro*, and *in vivo* studies [94]. Aflatoxin B is produced by *Aspergillus flavus* which is a common contaminant of peanuts, particularly in humid climates. Doses as small as 0.1 ppm in the diet induced HCC in 100% of Fisher rats after 54 weeks [48].

Alcohol

Alcoholics have a 3 to 7 times increased risk of HCC, although alcohol itself is not considered a carcinogen [95]. Instead, a number of mechanisms whereby alcohol could be a co-carcinogen have been proposed: one is that ethanol induces liver microsomal enzymes and in doing so may enhance the biotransformation of chemical carcinogens [95]. Another possibility is that ethanol promotes cancer through depression of the immune system [95]. Finally, the carcinogenic effects of ethanol may be related to the induction of cirrhosis [95].

RELATIONSHIP BETWEEN CIRRHOSIS AND HCC

While the precise relationship between cirrhosis and HCC is unknown, it is clear that an association exists between the two diseases. Some authors have suggested that cirrhosis is of itself a preneoplastic condition. Overall, patients with cirrhosis have a 10-40 times increased risk of developing HCC [51]. A number of hypotheses have been proposed to explain the increased incidence of HCC in cirrhotic patients:

Firstly, it has been observed that cirrhosis and HCC can be caused by the same agent or agents [96]. HBV infection, for example, is both a primary cause of cirrhosis and HCC worldwide. It has been observed that rates of HCC in various countries parallel the prevalence of HBV infection [97]. Other agents implicated in the causation of both HCC and cirrhosis include Aflatoxin B [94], ethanol [95], and HCV [96].

Secondly, cirrhotic livers have a high rate of cell turnover, which may contribute to the initiation of neoplasia. It is postulated that multiple cell divisions may be responsible for the initiation and promotion of HCC in cirrhotic livers [41,98]. With repeated mitotic activity, particularly over many years, the creation of mutations as well as the integration of exogenous DNA into the genome is facilitated [98]. In addition, similar oncogenes have been documented in both hepatocarcinogenesis and regeneration [99].

Thirdly, it has been suggested that cirrhosis may not act as an initiator of HCC but rather as a promoter, by increasing the susceptibility of the liver to environmental carcinogens [96]. Hepatocytes in the S phase are more susceptible to DNA alterations, because DNA is temporarily single-stranded. Also, rapid proliferation interferes with DNA repair processes [96]. While a direct link between nodular regenerative hyperplasia and HCC has not been definitely established, some authors believe that the process is a precursor lesion for HCC [100], particularly when associated with dysplasia [101].

Fourthly, distortion of the extracellular matrix (ECM) could be involved in the transition of cirrhosis to HCC [102]. The ECM plays an important role in regeneration and cell division. Collagen deposition leads to a distortion of the architecture and scaffolding, and as a result, microenvironmental changes have been documented in cirrhosis. These include loss of the lobular gradient, abnormal cell polarity, uncoupling of cells, and the closure of gap junctions [96], resulting in abnormal cell-to-cell communication, cell division, and processing of promoter and inhibitory signals.

The clinical data, on the other hand, suggest that cirrhosis alone is insufficient to produce HCC, and that additional cofactors are required. In high incidence populations such as African blacks, a large fraction of patients with HCC do not have cirrhosis, whereas in lower incidence populations such as North America, only a small fraction of patients with cirrhosis have HCC [96]. Similarly, whereas the incidence of HCC associated with HBV in Africa is as high as 50%, the risk of HCC with Wilson's disease or PBC is much lower [103].

Therefore, four mechanisms have been proposed whereby cirrhosis is associated with HCC, including similar etiologic factors, rapid cell turnover, increased susceptibility, and distortion of the ECM. It is likely that all mechanisms may be involved to some extent, each producing abnormal cell divisions, and predisposing to carcinoma.

TREATMENTS

Resection

In general, surgical removal of all the cancer offers the only chance for cure of HCC [104]. Yet a large proportion (40-85%) of the patients presenting with HCC are considered unresectable because of end-stage cirrhosis, invasion of structures in the portal area, or underlying medical conditions [2,3,105].

A formal lobectomy and clear margins of at least 1 cm are desirable in a patient with a normal liver [104], [106]. Nonetheless, even following such resections, the recurrence rate has been reported to be 45 % to 64 % within 3 years [107,108].

In a patient with cirrhosis and HCC, however, a formal lobectomy would in most cases place the patient at high risk of post-operative liver failure. Obtaining a margin of 1 cm may not be feasible. In fact, only a small number of patients with cirrhosis and HCC are operated on, with resectability rates of 13% and 17% reported [105,109]. A recent study of a large multicenter group of patients noted a resectability rate of 30% [110].

One retrospective study compared outcomes following formal lobectomy *versus* segmentectomy in 50 cirrhotic patients [111]. Early postoperative mortality was higher in the former group, while long term survival was similar at 1, 2, and 5 years, provided a 10 mm margin was obtained. The results of this study suggest that the procedure of choice for HCC in patients with poor liver function is segmentectomy with adequate margins.

A recent study examined the role of a clear margin following limited surgical resection. The study suggests that in cirrhotic patients with HCC and tumors under 4 cm in diameter that a 10 mm margin does not influence the rate of recurrence [107].

Favorable prognostic factors following resection include tumor encapsulation [112], fibrolamellar variant [15], and small size (< 3 cm) [105]. Cirrhotic patients with these characteristics should be treated aggressively, as even limited resection yielded an improved long-term survival [111].

It has been suggested that for comparable tumors and extent of resection that recurrence of HCC is higher in a cirrhotic liver than in a non-cirrhotic one. Sasaki and associates examined long-term survival in 142 patients with cirrhosis and 48 patients without cirrhosis. The main cause of death in both groups was cancer recurrence. Long-term survival at 5, 7, and 9 years was 44%, 32%, and 26% respectively in patients with cirrhosis, versus 68%, 57%, and 57% respectively in patients without cirrhosis. The authors suggest that the premalignant environment of the cirrhotic liver results in higher recurrence rates in cirrhotic patients [113].

For patients considered unresectable or poor surgical candidates, a number of treatments have been proposed for palliation, and occasionally, as adjuvants to surgical treatment. Nonetheless, surgical resection offers the only chance of a cure.

Systemic Chemotherapy

In general, systemic chemotherapy has shown disappointing response rates in the treatment of HCC. A phase II trial with Mitozanthrone documented a response rate of only 27.3% [114], as defined by a decrease in tumor size on CT scan. Systemic chemotherapy may be more useful postoperatively as an adjuvant agent [15], but this remains to be determined.

Intraarterial Chemotherapy

To increase the dose of chemotherapeutic agents delivered to the tumor, the hepatic artery may be cannulated surgically or percutaneously. Subcutaneous pumps have been described which allow for the prolonged, continuous infusion of chemotherapeutic agents. While response rates to intraarterial infusion of doxorubicin approach 50%, the superiority of intraarterial therapy over systemic therapy has not been convincingly established [115]. Furthermore, despite the response to treatment achieved with intraarterial chemotherapy, survival remains unchanged [115].

Embolisation

The principle of this treatment takes advantage of the hepatoma's dependence on arterial supply. Transarterial embolisation (TAE) involves the embolisation of 1 to 2 mm gelfoam particles, and is becoming widely used for treatment of non-resectable tumors as well as tumor recurrences.

Preliminary results with the technique appear promising, with good ischemic effect on CT scans and autopsies even in large tumors [116]. A

prospective trial is needed to determine the usefulness of TAE in palliation or as a neoadjuvant therapy.

The technique has some problems associated with its use, including embolization to the cystic artery and the splenic artery [117], [118]. Furthermore, the use of TAE is limited by the fact that embolisation in the cirrhotic liver carries the risk of liver failure if too large of a segment is rendered ischemic [119].

The application of this treatment requires that the portal vein be patent, otherwise obstruction of arterial and portal blood supply leads to frank infarction of the parenchyma [120]. The effect of TAE is limited by the formation of collateral vessels which develop within a few days of treatment [118].

Chemoembolisation

Chemoembolisation is a variation of the embolisation technique in which minute capsules of a chemotherapeutic agent such as mitomycin C are embolised into smaller arteries. The advantages of this approach are that chemotherapy can be applied locally, that embolisation occurs more distally in smaller arteries, and that delivery of the agent is sustained [121]. In one study which examined both cirrhotic and non-cirrhotic patients, a decrease in tumor size was seen in 30% of cases [122] and the three-year survival rate of 25% compared favorably to that seen in a comparison group which underwent resection. In another study, no advantage could be demonstrated from the use

of this approach over gelfoam embolisation [123]. Once again, a controlled clinical trial is needed to define the role of chemoembolisation.

Radiation

HCC has been shown in numerous studies in the past to be relatively unresponsive to radiation when used alone. It may be of some benefit when used in combination with chemotherapy or ethanol injections, but evidence in the form of clinical trials is lacking [48]. In addition, normal parenchyma and the gastrointestinal tract mucosa is subject to atrophy and microvascular damage with moderate radiation doses [109].

Ethanol injections

This modality, which has received much attention recently, involves the injection of absolute ethanol percutaneously into the tumor via a thin needle guided by ultrasound. Use of this technique is becoming more widespread in inoperable patients, because it is inexpensive and quick, and can be done under local anesthesia. Response rates of 30% or more have been observed [124]. Its disadvantages are that numerous sessions are required and that severe pain often occurs following the injections. The outer rim of malignant hepatocytes also remain viable, so that tumor recurrence is inevitable [124].

Treatment of HCC by transplantation

Initial results with transplantation for the treatment of HCC were dismal; survival in early series ranged from 0% 1 year survival [125], to 17% 5 year survival [126]. This is likely related to the presence of extrahepatic

micrometastases at the time of diagnosis [127]. In addition, immunosuppression may interfere with cancer defense mechanisms and predispose the patient to earlier recurrence [128]. One study documented markedly decreased tumor doubling times in immunosuppressed patients following liver transplantation, compared to patients who underwent resection for HCC [129]. Following these initial experiences, attempts at liver transplantation for HCC were for the most part abandoned. It was thought that liver transplantation for the treatment of HCC was futile [130], and that scarce donor organs should be used elsewhere.

Iwatsuki and Starzl reported on a series of patients transplanted for end-stage liver failure who were found to have incidental small HCC's in the explanted liver. These patients would be less likely to have extrahepatic micrometastases. No tumor recurrence was detected in 12 of 13 patients during a follow-up of 7 years (4 months to 15 years) [131].

Another study emphasized the importance of lymph node involvement in 57 patients with HCC in determining outcome following transplantation. All patients with positive nodes survived less than one year, whereas patients with negative nodes had survival rates of 42%, 36%, and 24%, at 1, 2, and 3 years following transplantation [132]. It became apparent that perhaps certain subgroups of patients could be identified for which liver transplantation could be beneficial.

LIVER RESECTION VERSUS TRANSPLANTATION

To date, the only curative treatment for hepatocellular carcinoma is complete surgical removal of the tumor, either by hepatic resection or total hepatectomy followed by liver transplantation. Early results of transplantation for HCC were very poor, however, in the past few years, some promising results have been reported, likely due to improved patient selection. A few retrospective studies have compared the two approaches allowing a few conclusions to be drawn, however, no prospective data are available, and a multicenter, randomized controlled trial is needed to further define the role of each treatment modality in the management of HCC.

Theoretically, liver resection would be expected to lead to a higher rate of local recurrence due to the presence of remaining preneoplastic cirrhotic tissue [113]. Conversely, while orthotopic liver transplantation should decrease the rate of recurrence, the immunosuppressed state has been shown to decrease tumor doubling time in any remaining extrahepatic tumor [129], and lead to a shorter time to recurrence. Clearly, the success of either approach depends upon ruling out the presence of extrahepatic metastases.

Iwatsuki and Starzl compared resection to transplantation in the setting of HCC [133]. Patients were stratified by TNM staging and it was found that survival correlated well with TNM stages for both groups. The study revealed similar 5-year survival for liver transplantation and resection (47.2% versus 35.6%, respectively), however, when HCC was associated with cirrhosis,

survival was better after liver transplantation at all stages. Unfortunately, the study was retrospective; while some useful observations could be made, a selection bias was present in that patients in the transplantation group were generally those who had worse liver function preoperatively.

Bismuth attempted to identify patients which would be more likely to benefit from transplantation [134]. Patients with tumors less than 3 cm and consisting of less than 3 nodules who were transplanted had markedly better survival and recurrence free survival compared to a group with similar patient characteristics who underwent resection (3 year survival 83% vs. 41 %, recurrence free survival 83% vs. 18%).

Another retrospective review examined a series of 198 patients with 131 undergoing resection and 61 undergoing transplantation [135]. Resection for HCC in patients without cirrhosis yielded better 3-year survival than those patients which underwent transplantation (45% vs. 11.8%, respectively). Again, due to the retrospective nature of the study, marked differences existed between the two groups. In particular, the transplantation group had a higher proportion of cirrhosis (57.4% vs. 17.6%) as well as higher tumor stage. Factors predictive of increased survival were identified by multivariate analysis for resection (age 30-50, absence of cirrhosis, fibrolamellar variant, solitary tumor, absence of vascular invasion), and for transplantation (absence of regional lymph node metastasis and distant metastasis).

Mazzoferro et al. examined 48 patients with cirrhosis who underwent liver transplantation for unresectable, but small HCC's (single nodule < 5 cm, or up to 3 nodules, each < 3 cm) [136]. Patients were judged to have unresectable disease if the location of the tumor was prohibitive, if the tumor was multifocal, or if advanced cirrhosis was present. Overall 3-year actuarial survival following transplantation was an impressive 75% which compares favorably with 3-year survival of untreated HCC of <25%. When the specimens were examined, differences were seen in the pathology results compared to the extent of disease which was predicted by preoperative assessment. Among the 35 patients who met the predetermined criteria for "small HCC" at the time of pathological examination, overall survival was 85%, whereas in the 13 patients whose tumors were larger than originally staged, 5-year survival was 50%.

A recent study focused on survival and recurrence in 102 patients following resection or transplantation [137]. Survival and rate of recurrence were not significantly different between the two groups, although there was a trend towards decreased recurrence in the group treated with liver transplantation ($p=0.067$). Univariate analysis revealed that tumor size > 3 cm was a significant risk for tumor recurrence. Here, too, differences existed between the groups treated with resection and transplantation. In particular, the group receiving a liver transplant tended to be younger, and contained a higher proportion of cirrhotic patients.

Whereas the results of transplantation for HCC were dismal initially, transplantation has been since shown to be an effective treatment when patients meet certain specific criteria (small tumors, cirrhosis, and tumors unresectable because of central position). Clearly, neither resection nor transplantation is effective for advanced HCC or extrahepatic disease. Currently, liver transplantation and liver resection should be viewed as two available approaches to surgical cure. To date, no prospective data exist and a randomized controlled clinical trial is needed to further determine the role of both approaches in the treatment of HCC, particularly in view of the limited supply of donor organs.

THE CLINICAL PROBLEM: HEPATIC RESECTION OF CIRRHOTIC LIVER

MORBIDITY AND MORTALITY

Hepatic resection in non-cirrhotic patients can be accomplished with relative safety, and a morbidity of well under 5% for elective lobar resections is commonly achievable in most centers. The main risk factor appears to be associated medical illness [138].

On the other hand, the risks and complications associated with resections in cirrhotic patients are more complex. The extent of resection and degree of baseline functional impairment appear to be the main independent risk factors. Other factors, such as bleeding tendency, ascites, anemia, and hypoalbuminemia appear to be dependent on these two variables [139,140]. The serious complications that these patients are prone to develop are hepatic failure, sepsis, and bleeding, which can all largely be attributed to insufficient hepatic function.

Cirrhotic patients are prone to develop these complications when undergoing surgery of any kind, not only hepatectomy. Doberneck examined cirrhotic patients undergoing a variety of operations including intraabdominal, genitourinary, and orthopedic procedures [141]. An overall complication rate of 47.1% along with a mortality rate of 19.6% was seen. Liver failure occurred in 43 patients, sepsis in 19 patients, and bleeding in 9 out of 102 patients.

In 1985, Yanaga noted intraperitoneal septic complications in 16 of 103 patients undergoing hepatic resection [142]. On the other hand, only 3 of 46 patients without cirrhosis developed sepsis. The cause of death in the 13 patients that died was noted as progressive liver failure.

Another large series identified a mortality rate of 8.9% and a complication rate of 31.7% among 101 cirrhotic patients undergoing major and subsegmental resections for HCC [143]. Sepsis was seen in 4 of 102 patients, bleeding in 10 patients, and liver failure in 10 patients. The lower mortality rate seen here can be explained by the fact that over half the resections were subsegmental and a large number were done for palliation only.

Fan and associates published results of a series of 54 cirrhotic patients undergoing major resections [144]. Seven deaths occurred, for a mortality of 13%, and serious complications were seen in 50%. Sepsis complicated the early postoperative course in 18 patients, while excessive bleeding was seen in 4 patients. No mention was made of the incidence of liver failure.

Most studies of resection of HCC are performed in Asia; nonetheless, two European studies have yielded similarly high morbidity and mortality rates. Bismuth reported on 35 of 270 patients with cirrhosis and HCC who proceeded to resection [2]. A mortality rate of 14.3% (5/35), and a morbidity rate of 47.2% (20 / 35) were reported despite a very carefully selected patient population.

Another European group, reported, in 72 patients, a complication rate of 49% during two months postoperatively, and a mortality rate of 7%. Limited resections (segmental or subsegmental) were carried out in 81% of the patients.

An important point to be made is that only a small fraction of patients with cirrhosis and HCC are considered operable. The rate of operability reported by Bismuth (13%), is only slightly lower than that reported in Japan by Okuda (17%) [109], and the Liver Cancer group (30%) [145]. Thus, the morbidity and mortality rates reported to date apply to carefully selected patients which serves to emphasize the hazardous nature of resecting even small amounts of liver mass from cirrhotic patients.

COMPLICATIONS

Hepatic Failure

The term "hepatic failure" does not have a precise definition, and as a result, the reported incidence of this complication varies widely. Liver failure is, however, consistently quoted as the most common cause of death in cirrhotic patients early in the postoperative period. One author has proposed that hepatic failure includes progressive jaundice, ascites, and encephalopathy, in addition to rapidly deteriorating liver function studies [146]. Liver failure can also result in insufficient metabolic, synthetic, and immune function, predisposing to the other complications such as encephalopathy, jaundice, hypoalbuminemia, hypoglycemia, and sepsis.

If, after estimating post-operative liver function, it appears that insufficient liver function will remain, it is essential to reduce major resections, by considering segmentectomy or even subsegmental resection.

Sepsis and ARDS

Cirrhotic patients undergoing resections are predisposed to infection, both locally and systematically. This could be related to the loss of hepatic reticulo-endothelial system function due to resection [146].

In one study of 149 patients undergoing resections, most of whom were cirrhotic, perioperative variables associated with septic complications were examined [147]. Risk factors identified included right lobectomy, age greater than 65 years, operation time greater than 5 hours, blood loss greater than 3000 mL, and postoperative bleeding requiring reoperation. Notably, the risk of sepsis did not correlate with preoperative liver function tests nor with the presence of cirrhosis. The authors postulate that this relates to the fact that more limited resections were carried out in cirrhotic patients.

The liver's role in filtering blood prior to entry into the lungs may in part explain the increased risk of ARDS following surgical resection of cirrhotic livers [146].

Bleeding

The factors predisposing the cirrhotic patient to bleeding are multiple and have been studied extensively. Amongst these are decreased hepatic synthetic function, which leads to low levels of clotting factors. The result is an abnormal

PT / PTT. Due to the short half life of clotting factors (most under one day), PT is a sensitive indicator of acute changes in liver synthetic function [148].

Portal hypertension is also a factor in increased bleeding and collateral neovascularisation makes dissection difficult, particularly in the area of the porta hepatis. The classic paper by Schwartz in 1981 describes massive bleeding in 18 of 33 patients who underwent cholecystectomy or an operation for bile duct obstruction, despite the presence of normal coagulation profiles in approximately half the patients [149]. On the other hand, among patients undergoing cholecystectomy at the same time as a portal decompressive procedure, significant bleeding occurred in only one of seven patients [149]. These findings emphasize the role of elevated portal pressure in contributing to the bleeding associated with biliary tract surgery. A number of studies have examined the role of intraoperative vasopressin as a means of decreasing portal pressure and thereby limiting perioperative bleeding problems. During shunt operations, Sirinek et al. reported a 28% decrease in portal pressure with vasopressin, which was associated with a decrease in blood loss, and a shorter operating time [150].

Over the last 20 years, the existence of a fibrinolytic state as well as the extent of its contribution to the problem of operative and postoperative blood loss has been debated. When properly diagnosed, bleeding associated with a fibrinolytic state has been successfully controlled with the careful use of aminocaproic acid [149]. Another antifibrinolytic is aprotinin (Trasylol®), a

serine protease inhibitor which inhibits plasmin and kallikrein. It has been used successfully as an antifibrinolytic agent in cardiac surgery and liver transplantation [151], and may similarly be beneficial to cirrhotic patients undergoing resection.

It is important to correct hemostasis preoperatively and to monitor hemostatic function closely postoperatively. Recent advancements in critical care have contributed significantly to the decrease in bleeding complications in this setting [152].

OTHER FACTORS

There are a number of factors that make it difficult for a cirrhotic patient to tolerate resection, including poor functional reserve, impaired regeneration, elevated portal venous pressures, and the characteristics of the patient population undergoing resection.

Reduced Function

The removal of functional liver tissue from an organ that already has marginal function can lead to many complications. If insufficient functioning hepatic parenchyma remains after resection, the patient will likely develop liver failure in the postoperative period. Yet it is crucial to be able to resect a sufficient amount of liver to effect a cure, while leaving enough healthy liver to ensure survival. A minimum amount of liver function is essential to sustain life.

Impaired Regeneration

Unlike the normal liver, which, even after 80% resection, can regenerate to its original size and function within 3 to 12 months, the cirrhotic liver has impaired regenerative ability [7]. Indeed, until recently, the cirrhotic liver was thought to be unable to regenerate at all [6]. However, it is now accepted that while it is able to regenerate somewhat, the cirrhotic liver is greatly impaired both in terms of rate and extent of regeneration [7]. The reason for this impaired regenerative capacity is currently the subject of numerous investigations.

Portal Hypertension

Portal hypertension poses a number of problems in cirrhosis. Principal amongst these is that the rate of blood loss is accelerated and hemostatic mechanisms are often unable to overcome venous bleeding because of the high pressures involved. In addition, numerous venous collaterals are formed in an attempt to bypass the high resistance of the intrahepatic vasculature. These collaterals are particularly prominent in the area of the porta hepatis, making dissection difficult and hazardous. This likely contributes to the high mortality rate noted in the performance of cholecystectomy in cirrhotic patients [148,149].

TREATMENTS EXAMINED IN THE STUDY

CIPROFLOXACIN

Ciprofloxacin is a quinolone antibiotic effective against a wide variety of gram negative bacteria as well as members of the *Staphylococcus*, *Enterobacteriaceae*, and *Pseudomonas* families of bacteria [153]. This antibiotic is currently used in clinical practice in the treatment of urinary tract infections, as well as respiratory tract infections, gonorrhea, and sepsis associated with liver failure.

STRUCTURE

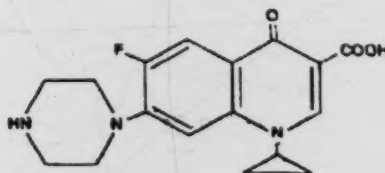


Figure II : Structure of ciprofloxacin

ABSORPTION

Following oral dosing, ciprofloxacin is readily absorbed in the duodenum and jejunum [154]. Bioavailability following oral dosing is excellent, from 66-88% [153], and absorption is unaltered by food. Protein binding is considered to be negligible [153].

METABOLISM AND EXCRETION

Ciprofloxacin is partially metabolized in the liver to at least four metabolites [155] with over 60% of ciprofloxacin excreted unchanged in the urine [155].

Elimination $t_{1/2}$ is approximately 5 hours [153].

Two studies have examined the effect of liver failure or cirrhosis on ciprofloxacin pharmacokinetics [155], [156]. In cirrhotic patients, the peak serum concentration is slightly increased, however, elimination $t_{1/2}$ is not significantly increased unless coexisting renal failure is present. The authors conclude that dosage adjustment is not required in patients with cirrhosis and normal renal function.

DOSE

The recommended dosage in humans is 250 - 500 mg orally, twice to three times daily [157]. A toxicology study examined the effects of doses of 20 mg / kg and 200 mg / kg per day in rats [158]. At the higher dose, significant chromatid strand breakages were seen as well as increased urine volume, creatinine, and decreased creatinine clearance, compared to saline controls. The dosage of 200 mg / kg / day appears to exceed the optimal therapeutic dose.

ACTIONS

Ciprofloxacin is a quinolone antibiotic, and its antibacterial effect is thought to be due to inhibition of bacterial DNA topoisomerases [159], with negligible effects on eukaryotic DNA enzymes [160].

In addition to its antibiotic actions, ciprofloxacin possesses intrinsic GABA-receptor blocking properties [161]. GABA receptors have been identified on the surface of hepatocytes [162], and activation of these receptors inhibits hepatic regenerative activity [163]. Specifically, GABA administered to rats following 70% partial hepatectomy resulted in significantly decreased restitution of liver mass, protein synthesis, ornithine decarboxylase activity, and putrescine levels. Moreover, GABA receptor antagonists reverse the inhibitory effects of ethanol on hepatic regeneration [163]. These data provided a starting point for further investigations of the GABA receptor blocking and regeneration promoting properties of ciprofloxacin in cirrhosis. These studies of the effect of GABA or ciprofloxacin on regeneration have all been done in a setting where resection is used as the regenerative stimulus, and so the results are applicable to the clinical situation addressed in this thesis. The effect of resection on the expression of the GABA receptor, however, will have to await the cloning of the GABA receptor gene which is currently underway in our laboratory.

The importance of the gut flora and inhibitory factors produced and absorbed in the colon raise the possibility that the beneficial effects of ciprofloxacin could be due to changes in the gut flora. A study examining the

effect of norfloxacin, a quinolone antibiotic which has low bioavailability and has negligible GABA receptor blocking activity [164], showed no effect on hepatic regenerative activity. These data suggest that ciprofloxacin's beneficial effects on liver regeneration are likely independent of its effect on gut flora [165]. The GABA receptor blocking ability of some quinolone antibiotics possibly relates to the presence of the piperazine moiety at the 7 position of the parent ring. Norfloxacin has a methyl group substituted for the piperazine group [164].

The inhibitory effect of ethanol on liver regeneration is well known. Although the mechanism remains unclear, ethanol is a potent stimulator of GABA receptor activity. With this understanding, one study compared groups of rats given saline or ciprofloxacin 100 mg / kg one hour before an oral dose (5 g / kg) of ethanol. A protective effect was seen in the group pretreated with ciprofloxacin. This group did not manifest the inhibition of regeneration that was seen with ethanol, as measured by ^3H -Thymidine incorporation and PCNA index [166].

Another study [167], examining a fulminant hepatic failure model in the rat, documented a protective effect of ciprofloxacin on the inhibition of regeneration seen in this disease. Also, a dose of 100 mg / kg / day appeared optimal, while 250 mg / kg / day appeared deleterious.

A recent study examined the effects of ciprofloxacin on the liver in a CCl_4 - induced rat model of cirrhosis [168]. After a four-week course of treatment with ciprofloxacin 50 mg / kg twice daily, fibrosis, as determined by histology and

levels of proline and hydroxyproline in the liver, was significantly decreased, as was inflammation, as measured by serum ALT levels. Hepatic regenerative activity was not examined.

Thus, previous studies have shown that ciprofloxacin enhances hepatic regeneration, decreases fibrosis, and is safe in the setting of advanced liver disease. Such effects may be useful to prepare the cirrhotic liver to better tolerate hepatic resection. Furthermore, in rats, a dose of 100 mg / kg / day appears to be effective.

PENTOXIFYLLINE

Pentoxifylline is a methylxanthine derivative type IV phosphodiesterase inhibitor which initially received attention as a blood flow altering agent useful in patients with peripheral vascular disease. More recent studies have identified numerous other possible uses of the drug.

STRUCTURE

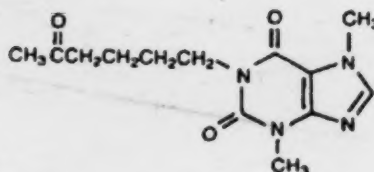


Figure III : Structure of pentoxifylline

ABSORPTION

Absorption of pentoxifylline after oral administration occurs readily, and virtually complete absorption of oral doses has been confirmed by comparison with absorption following IV administration [169]. Absolute bioavailability was calculated at 30.5% [169]. No significant degree of plasma protein binding can be demonstrated which is potentially relevant to the hypoalbuminemia state seen in patients with cirrhosis [170].

METABOLISM AND EXCRETION

Metabolism occurs primarily by reduction to form the pharmacologically active metabolite 1, hydroxypentoxifylline. Seven other metabolites have been identified [171]. Excretion occurs via the kidneys and patients with reduced creatinine clearance have been shown to have markedly decreased clearance of pentoxifylline [171]. In healthy subjects, elimination $t_{1/2}$ was 0.89 hours [169]. The principle active metabolite has a $t_{1/2}$ of 0.97 hours.

One group examined the pharmacokinetics in histologically proved cirrhotic patients with Child-Pugh² scores in the 5-9 range for intravenous dosage and oral administration [172]. Following intravenous administration, the $t_{1/2}$ more than doubled from 0.83 hours in healthy volunteers to 2.12 hours in cirrhotic patients. $T_{1/2}$ could not be calculated for the sustained release (SR) oral formulation because of prolonged absorption, but peak plasma levels of pentoxifylline were markedly higher in cirrhotics (413 versus 55 ng/ml). In humans, only the SR formulation is used, however this depends on administering the drug in an encapsulated form – a liquid form is required for rat studies [172].

² The Child-Pugh system is a scoring system which was originally devised to assess the prognosis of cirrhotic patients undergoing portal-systemic shunts. A score of 1 to 3 is assigned for each of 5 categories, including degree of ascites, degree of encephalopathy, serum bilirubin, serum albumin, and PT INR. Higher scores indicate a worse prognosis.

MECHANISM OF ACTION

While pentoxifylline was primarily designed as a phosphodiesterase inhibitor, many other actions have been documented. Pentoxifylline has been shown to have blood flow altering effects, with actions on erythrocyte deformability, blood viscosity, platelet aggregation, and plasma fibrinogen concentrations [171]. Such hemorrheologic effects make the drug useful in peripheral vascular and cardiovascular disease. Another well studied action is the inhibition of $\text{TNF-}\alpha$ by pentoxifylline both *in vitro* [173] and *in vivo* [169].

The reason for choosing pentoxifylline for use in this study is its documented effect of inhibition of Platelet Derived Growth Factor (PDGF) - dependent fibroproliferation. An *in vitro* model was devised by Peterson to measure fibroproliferation as assessed by ^3H -Thymidine in fibroblasts. The study assessed several agents for their ability to block the fibroproliferative effect of PDGF. Pentoxifylline effectively blocked PDGF dependent fibroproliferation at all doses studied [174]. A similar effect was noted in another study by Peterson, where pentoxifylline was effective in blocking PDGF induced fibroproliferation in serum samples taken from patients with liver fibrosis [22].

Another *in vitro* study suggests that pentoxifylline also decreases the differentiation of Ito cells to myofibroblasts. This effect may be related to inhibition of the PDGF receptor [17].

The precise mechanism whereby pentoxifylline interferes with PDGF requires further study. In one study, it was noted that the effects of pentoxifylline were comparable to those of trapidil, a known reversible competitive antagonist of the PDGF receptor. The results suggest that pentoxifylline affects the PDGF receptor or PDGF dependent signaling [71]. Further studies with adenosine suggest that adenosine alone was a more potent inhibitor of PDGF, which suggest that post-receptor effects may also be at work [175].

Many animal and human trials have been performed using pentoxifylline demonstrating effects on the microcirculation. Only one study, however, has been performed in an animal model of liver fibrosis. The model used by Peterson was the yellow phosphorus-induced pig model. The study showed that a 12-week course of pentoxifylline 16 mg / kg daily significantly reduced liver fibrosis as assessed by morphometry, and improved functional abnormalities as measured by γ -glutamyl transpeptidase (γ -GGT) and alkaline phosphatase. [176]. The effects of pentoxifylline in a rat model have yet to be reported.

SIDE EFFECTS

With the use of pentoxifylline, there exists a therapeutic window of serum concentrations where the drug is effective and where higher doses produce toxicity [171]. The side effects most commonly reported in humans include gastrointestinal disturbances such as nausea and diarrhea [171]. Other more serious abnormalities such as dysrhythmias and seizures occur much more rarely [171].

The well-studied mechanisms and effects of pentoxifylline, along with its relative safety during clinical use for other conditions, suggest that pentoxifylline may be useful clinically to decrease fibrosis and improve outcome following liver resection in cirrhotic patients.

TRADITIONAL CHINESE HERBAL REMEDY

For centuries, Chinese herbal remedies have been used in the treatment of liver disease. While many formulations exist, few have been well studied in a controlled scientific fashion. One specific formulation contains several ingredients, including *parowax* root, leeches, *pangolin* scales, and ginseng. Norman Bethune University has a patent pending on the precise formula, so a detailed summary of the method of preparation of the remedy cannot be listed here. This specific formula was found in a prospective, controlled trial to have a beneficial effect on the extent of hepatic fibrosis in a rat model of cirrhosis [168]. In fact, fibrosis and architectural distortion appeared to be reversed almost to normal. This report represents the only reference to this remedy in the formal scientific literature.

The mechanism of action involved and which of the components is active is unknown. Traditional Chinese herbal physicians state that it is a balance between all the ingredients, rather than one particular ingredient which is necessary for the therapeutic effects [177]. Others state that the formulation, in particular the ginseng component, is extremely nutritious, and this may account for its helpful effects [178]. One problem with herbal remedies, including those available commercially, is that there is a lack of standardization with respect to potency and dosage. This problem is related to the fact that, in most cases, the active component is not known. In this study, the dosage of 1 cc / 200 g body

weight was administered to each rat during the treatment phase, as this dose was effective in Zhang's study.

In this early phase of testing the remedy, the objective is to demonstrate whether or not a consistent beneficial effect is associated with using the remedy.

Future studies could examine which component or components are active.

Much more study is needed in the area of this and other herbal remedies.

OVERVIEW OF THE STUDY

Rats with cirrhosis induced by CCl_4 were randomized to one of 4 study groups. Each group received treatment (ciprofloxacin, pentoxifylline, or TCHR) or saline (disease controls) for 4 weeks prior to undergoing partial hepatectomy. Liver function, regeneration, and fibrosis were analyzed as the outcomes of interest.

HYPOTHESIS

Preoperative treatment with ciprofloxacin, pentoxifylline, and TCHR will be useful in improving the outcome of hepatic resection, in terms of function, regeneration, and fibrosis, in this rat model of cirrhosis.

STUDY DESIGN

MODEL OF CIRRHOSIS

A number of animal models of cirrhosis have been proposed, including carbon tetrachloride (CCl_4) [6], yellow phosphorus [179], dimethylnitrosamine (DMNA) [180], and thioacetamide [180].

Although excessive ethanol consumption is a common cause of liver disease in humans, ethanol alone in a rat model does not produce cirrhosis [181]. One study looked at groups of rat who were fed 36% of their daily caloric intake in the form of ethanol [181]. This regime, after one year resulted in fatty liver, however there was no evidence of hepatitis or cirrhosis. A similar model

applied to baboons, produced cirrhosis in all animals in less than one year [181].

The reasons for the different hepatotoxicity of ethanol between species is not clear, but may relate to the lack of production of inflammatory changes seen in the rat.

By far, the model which has been used most often and whose characteristics have been extensively documented is the CCl_4 -induced cirrhotic rat model. CCl_4 is metabolized by hepatocyte microsomal enzymes, including the P450 system, to the trichloromethyl ($\text{CCl}_3^{\bullet}$) free radical. This short-lived but very active free radical is capable of causing liver damage by lipid peroxidation and reaction with surface proteins. Since the free radical is short-lived and CCl_4 requires activation by the liver, the damage caused by CCl_4 administration is quite specific for the liver [182]. To enhance the efficiency of production of $\text{CCl}_3^{\bullet}$, and hence liver damage, induction of the microsomal enzymes using phenobarbital or ethanol has been proposed and is often utilized [183].

The key to inducing cirrhosis with this model is that repeated non-lethal insults to the liver must be delivered (at least 10 - 12), but between acute insults, the liver must be allowed to recover sufficiently to tolerate the next insult [180]. On the other hand, if CCl_4 administration is delayed or prematurely terminated, the liver has the chance to reverse the cirrhotic changes. Thus, to maintain cirrhosis, CCl_4 injections must be continued up to the time of surgery.

The CCl₄ model of cirrhosis has been used for over 50 years. Two groups have recently reexamined the model with respect to its validity as a model of human cirrhosis [184,185]. Both groups documented histological features and functional disturbances such as insulin resistance, and increased serum bilirubin and ammonia similar to the changes found in human cirrhosis.

MODEL OF RESECTION

The 70% hepatectomy in the rat is the classic model of hepatic resection first described by Higgins and Andersson in 1932 [29]. There are a number of reasons for using this popular model:

Firstly, the rat liver is anatomically ideal for performing quick, simple lobectomies. The four lobes (right, median, left lateral, and caudate) are distinct from each other, and each lobe tapers off near the porta hepatis making it amenable to being tied off at the base. The median and left lateral lobes are the most anterior and are mobile enough to be delivered easily into the incision. A 70% hepatectomy involves tying off those two lobes and amputating them with scissors. In the original description of the procedure, the two lobes constituted 70% of total liver mass, however, various authors have since reported percentages of 61 - 70 %, particularly in disease states [78], [185].

Secondly, it appears that a 70% hepatectomy is the optimal percentage needed to produce a significant regenerative stimulus. Studies performed in 1964 suggest that lesser extents of partial hepatectomy (10, 35, and 43 %) are capable of producing only negligible regenerative stimuli compared to 70 %

hepatectomy [186]. Greater extent of hepatectomy (90%) is often lethal, and has been used by some as a model of acute liver failure [187]. Moreover, we have shown that decreased rather than increased hepatic regeneration may occur after such extensive resections [188].

One potential problem with the 70% partial hepatectomy technique is that the percentage resected by performing a resection of the median and left lateral lobes varies with the species of rat used and the state of the liver. A difference of as little as 10% of liver mass resected can have a significant impact on the regenerative stimulus produced [188,189].

TIMING OF OUTCOME ASSESSMENT

The principle outcome variables (survival, post-operative liver function, and regeneration) were studied at 24 hours post partial hepatectomy because morbidity and mortality rates, deteriorations in liver function, and peak hepatic regenerative activity tends to occur within this time frame [83,190]. While regenerative events begin almost immediately, DNA synthesis, which is central to regeneration does not peak until around 24 hours post-resection [27].

Changes in the extent of hepatic fibrosis induced by the various therapeutic interventions would be apparent at the time of partial hepatectomy in the resected liver.

Initially, we planned to examine the outcomes at 24 and 48 hours following surgery. However, due to the small number of animals remaining in the cirrhotic control group (9), only one time point (24 hours) was used. While regeneration

at 24 hours should be sufficient, it would have been preferable to have two time points, in order to avoid missing delayed peaks.

SURVIVAL

As per the recommendations of the Canadian Council on Animal Care (CCAC), animals were sacrificed humanely when specific irreversible end-points were met, to minimize the period of pain and suffering preceding the eventual death of the animal [191]. The specific predetermined criteria for sacrificing the rats used in this study included: 1. Obtundation, unresponsiveness to being handled, to the other rats, or to toe pinch. 2. Visible jaundice 3. Any animal judged to be suffering unnecessarily for whatever reason. 4. In the postoperative period, any animal which had not progressed to stage 2 of the CCAC post-operative recovery guidelines [191] ("Animal can stand and move about") by 8 hours post operatively. These decisions were largely made by technicians at the animal care facility, who were blinded to the treatment groups.

LIVER FUNCTION

While a number of tests have been proposed as assessments of liver function, few are valid or practical for clinical use. In an animal model such as this one, choices are even more restricted. The classic liver function panel, which includes albumin, bilirubin, and prothrombin time (PT), continues to be the most popular means of assessing liver function, despite the development of other sophisticated tests [192]. All of the additional tests measure one or more

of the liver's many roles, such as blood flow, synthesis, metabolism, storage, and filtration by the reticuloendothelial system (RES). It is unclear which of the liver's many functions is the most relevant to an assessment of liver function. Moreover, other assessments of function such as indocyanine green (ICG) or aminopyridine clearance, involve the injection or inhalation of tracers, as well as multiple blood samples to plot a kinetic curve of elimination [193], which can be problematic in small animals with limited effective circulating volumes.

In clinical practice, hepatic synthetic function is often estimated using the prothrombin time (PT), which indirectly assesses the function of vitamin K dependent factors II, VII, IX, and X, as well as fibrinogen [194]. While this is a quick and simple test which provides useful prognostic data clinically [48], both ciprofloxacin and pentoxifylline may affect the PT and lead to deceiving estimates of liver function [193]. In the case of ciprofloxacin, this and other antibiotics have been documented to alter the bacterial flora and decrease vitamin K absorption, leading to an increased PT with prolonged use [193]. Likewise, pentoxifylline's many effects on blood flow include decreased fibrinogen levels which may account for some of its anti-thrombotic effects [171].

Although not a test of liver function *per se*, aspartate aminotransferase (AST) is an enzyme contained within hepatocytes involved in catalyzing the conversion of aspartate to pyruvate in the urea cycle [195]. Increased levels of this enzyme in the serum are a reasonable reflection of the extent of damage to hepatocytes. Alanine aminotransferase (ALT) is a similar enzyme used as an

index of hepatocyte damage. In cirrhosis, AST levels are higher than ALT levels; this is thought to be related to the hypoxemic injury secondary to intrahepatic vascular shunting.

Serum bilirubin remains a fair assessment of liver dysfunction, particularly in the absence of biliary obstruction or hemolysis [192,193]. A number of clinical studies have documented the importance of serum bilirubin as a prognostic factor for patients undergoing liver resection [196], or patients with liver disease undergoing surgery [197].

Serum bilirubin and AST levels were not drawn prior to surgery because of concerns regarding trauma induced mortality related to obtaining blood samples in hypocoagulable animals. Approval by the animal ethics committee was partly contingent on avoiding drawing blood samples preoperatively.

ASSESSMENT OF REGENERATION

A number of methods have been used to quantify cellular proliferation or regeneration in the liver. Some of the more common methods include calculation of liver weights, mitotic figure counts, ^3H -Thymidine incorporation, thymidine kinase and ornithine decarboxylase activity, and PCNA assays.

Liver Mass: While this is a somewhat insensitive measure of regeneration, an increase in liver mass is ultimately the end result of the regenerative process. Typically, in the rat much of the gain in liver mass occurs within the first few days, with further weight gains occurring at a slower rate for a few weeks thereafter [81].

³[H]-Thymidine: This is the method which is most commonly used in the literature. Briefly, tritiated Thymidine is injected intraperitoneally, 1 hour prior to sacrifice. The amount of ³[H]-Thymidine that is subsequently incorporated into liver DNA over 1 hour is considered a reflection of DNA synthesis and a parameter of regeneration [198].

The earliest studies examining ³[H]-Thymidine uptake as a measure of DNA synthesis in rat liver showed that only 1.2% of a 50 μ Ci dose of ³[H]-Thymidine is incorporated into DNA and that most of the incorporation was complete by 10 minutes [198]. At the end of the hour, all the thymidine is cleared [199]. It is not known how cirrhosis affects uptake and clearance of the tracer, however. In particular, shunting of blood flow around the liver such as occurs with portal hypertension could potentially lead to decreased uptake. Meanwhile, decreased breakdown of ³[H]-Thymidine by abnormal hepatocytes could lead to a falsely elevated measurement of ³[H]-Thymidine uptake.

Other disadvantages of the assay are that a radioactive tracer must be given and liver tissue is needed for analysis which usually involves sacrificing the animal. Finally, distinction between parenchymal and non-parenchymal cell uptake cannot be made. For these reasons, many investigators advocate the use of an additional, intrinsic index of regeneration in combination with ³[H]-Thymidine to obtain a more valid estimate of regeneration [200].

Thymidine kinase (TK): TK is a rate-limiting enzyme in DNA synthesis which is involved in the phosphorylation of thymidine prior to its incorporation

into DNA. Measurements of TK in liver tissue homogenates have been shown to accurately reflect increased DNA synthesis, and hence regeneration [201]. This assay cannot be used in conjunction with the ^3H -Thymidine assay as the latter interferes with the TK assay.

Ornithine decarboxylase (ODC): Both DNA synthesis and the production of growth-promoting polyamines depend upon ODC enzyme activity. Increased ODC tissue levels correlate with ^3H -Thymidine incorporation [202], while inhibition of ODC is associated with reduced regeneration [81]. ODC activity in liver tissue is an accepted surrogate marker of regeneration and is often used to complement ^3H -Thymidine in the quantitation of regeneration.

Proliferating cell nuclear antigen (PCNA): The PCNA molecule is a 36 kD nuclear protein that appears in late G1, and peaks in the S phase of the cell cycle. Elevated levels of PCNA correlate with cell proliferation [203]. The PC-10 monoclonal antibody is useful to quantify PCNA via immunohistochemistry, and the results correlate with ^3H -Thymidine and TK levels [204]. Unfortunately, like other immunohistochemical techniques, this approach suffers from interobserver variability due to the subjective nature of determining which degree of staining determines a 'positive' nucleus. Recent data from our laboratory indicate that PCNA protein levels determined by Western Blotting (using the PC-10 antibody) is a more quantitative and less subjective measure of liver regeneration [200].

QUANTITATION OF FIBROSIS

As discussed above, fibrosis consists of an increase in ECM components, particularly collagen types I and III in response to hepatic injury. Collagen type III is initially deposited, and replaced later by collagen type I [205]. Collagen deposition is the net result of fibrogenesis and fibrinolysis. Collagen is found in high concentrations even in healthy tissues, and the rate at which new collagen is being formed is difficult to measure. The level of procollagen type I mRNA transcripts is useful as an index of ongoing fibrinogenesis. Serum levels of procollagen type III amino-terminal peptide are currently used clinically as an index of ongoing fibrogenesis [206]. Unfortunately, these kits are expensive and repeated blood sampling is necessary.

Levels of MMP mRNA obtained by probing Northern blots can be used as a measure of fibrinolysis. To date, functional assays of MMP activity have been fraught with many difficulties due to cross reactivity. These problems can be circumvented by quantitating MMP production at the transcriptional level, and MMP mRNA activity has been useful as a measure of fibrinolytic activity [206,207].

In addition to these dynamic markers of fibrogenesis and fibrinolysis, the extent of fibrosis can be quantified using a number of methods. Since every third amino acid in collagen is proline, tissue proline or hydroxyproline levels can be used to estimate the degree of fibrosis [208]. A more rapid method, however, involves the use of computer assisted image analysis [209]. This involves

staining collagen bright violet using the Van Gieson stain, resulting in a sharp contrast between the collagen and the surrounding hepatocytes. A "fibrosis index" is calculated as the ratio between the area stained and the total area of the tissue in the visual field. Five visual fields per specimen were chosen at random for assessment.

Computer assisted image analysis has many uses in pathology and histology [210]. It has been shown that a strong correlation exists between this "morphometric" method of calculating the area of stained collagen and hydroxyproline quantitation [211].

METHODS

ANIMALS

One hundred and sixteen outbred male Sprague-Dawley rats weighing 200-250 grams (Charles River Canada, Trois Rivières, Quebec) were housed under standard conditions, including daily cycles of 12 hours darkness alternating with 12 hours light. Animals were weighed weekly to adjust dosages of CCl_4 and treatment drugs. The project was reviewed and approved by the University of Manitoba animal ethics committee.

CIRRHOSIS MODEL

Twelve healthy controls (HC) received no CCl_4 , ethanol, or treatment drugs throughout the study. In the remaining 104 rats, cirrhosis was induced by intraperitoneal injections of carbon tetrachloride (CCl_4 , Sigma, St. Louis, Mo.) mixed 1-to-1 with olive oil³ [212], at a dose of 0.3 mL / 100 g body weight, given twice weekly over 6 weeks. Ethanol (15% v/v) was added to the drinking water to enhance the effect of the CCl_4 [183].

TREATMENT GROUPS

The 64 CCl_4 induced cirrhotic rats that survived the 12 injections were randomized by computer to receive 1 of 4 treatments given daily by gavage over

³ Regular Olive Oil = saturated fatty acids 13 %, monounsaturated 74%, polyunsaturated 13%, linoleic acid = 12%

4 weeks. For the first 2 weeks, light ether anesthesia was required prior to gavage to reduce the risk of esophageal perforation or disruption of esophageal varices. Cirrhotic controls (CC) received normal saline by gavage at a dose of 1 cc per 200 grams, a volume identical to that administered to the other groups. The second group of cirrhotic rats received ciprofloxacin (Miles Canada, Etobicoke, Ontario) 100 mg / kg daily by gavage for the first two weeks of the treatment period, followed by 50 mg / kg twice daily for the last two weeks, when they were more amenable to the gavage process. The third group of cirrhotic rats received pentoxifylline (Sigma, St. Louis, Mo.) 32 mg / kg daily by gavage. The final group of cirrhotic rats received 1 cc / 200 grams body weight of a concentrated formulation of a traditional Chinese herbal remedy (TCHR), which contains ginseng, pangolin shell, and leeches as the principal ingredients [77]. The herbal ingredients were obtained from a local Winnipeg herbal medicine store. The dosages selected were based on the results of previous studies using these agents in rat models of advanced liver disease [20, 77, 166]. CCl₄ injections were continued weekly during this treatment phase of the study to prevent spontaneous reversion of the cirrhosis.

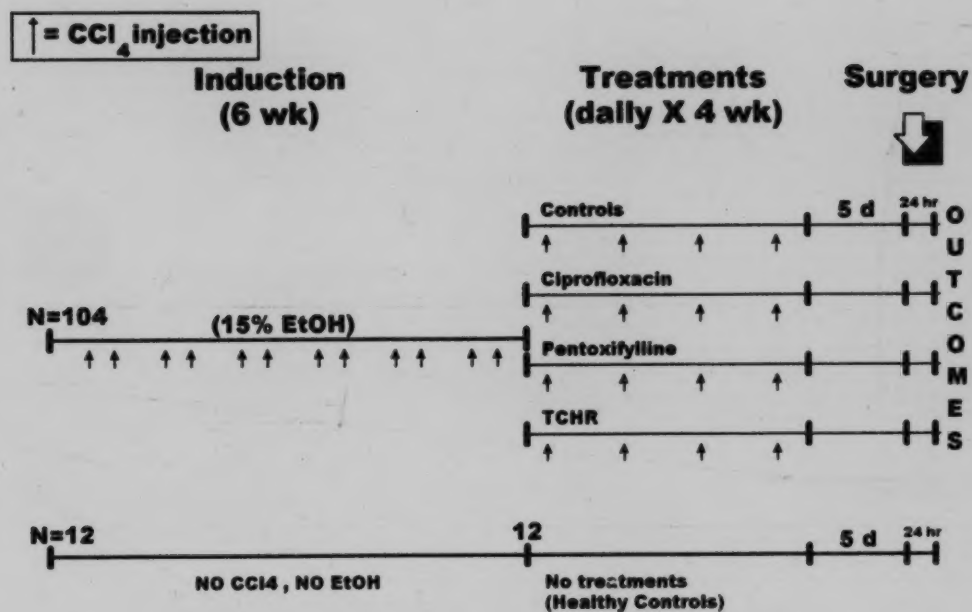


Figure IV: Summary of the experimental protocol

SURGERY

Over a five-day period preceding surgery, ethanol was gradually withdrawn from the drinking water of all groups because of its known inhibitory effect on hepatic regeneration [82], and CCl₄ injections were held to allow for acute inflammatory changes to subside. Because we were interested in the use of the interventions to prepare the cirrhotic liver for surgery, rather than the effect of the interventions at the time of surgery, drug treatments were not given on the day prior to surgery. For consistency with respect to perioperative nutrition, the animals were fasted for 18 hours before surgery.

Seventy-percent hepatectomies were performed by the method described by Higgins and Andersson [29] under general anesthesia using Isoflurane (Ohmeda Pharmaceutical, Mississauga, Ontario). The animals were coded such that the surgeon was blinded with respect to the group of the rat being operated on. A biopsy was taken for histology from the 70% hepatectomy specimen obtained and immersed in 10% formalin, while the remainder of liver tissue was rapidly frozen in liquid nitrogen and stored at -70 °C until needed for analysis.

Postoperatively, the rats were allowed free access to a 20% solution of sucrose in water solution. One hour prior to sacrifice, each rat received an intraperitoneal injection of ³[H]-Thymidine, 10 µCi per 200 grams body weight. Immediately prior to sacrifice by exsanguination under general anesthesia, a blood sample (5-10 mL) was collected by cardiac puncture. The remnant liver

was collected, weighed, and snap frozen to -70°C in liquid nitrogen. Samples were stored at -70°C until batch analysis could be performed.

All animals underwent post-mortem examination. In the presence of jaundice or ascites, and in the absence of complications from gavage (aspiration or mediastinitis from perforation), the cause of death was attributed to liver failure. Of note, three rats in the TCHR group died as a result of aspiration of the TCHR which was thick and viscous, and were excluded from the survival analysis. Warming the solution to 65°C using hot water greatly increased the ease of administration, and as a result no further deaths occurred in this group due to aspiration.

Table 1 : Measures used in the assessment of outcome

Survival
Function • serum AST • serum bilirubin
Regeneration • liver weights • ^3H-Thymidine incorporation • PCNA protein quantitation
Fibrosis • computer-assisted quantitation of fibrosis • Ludwig's fibrosis grade • procollagen and MMP mRNA expression

LIVER FUNCTION DETERMINATIONS

Serum AST and Serum Total Bilirubin

Serum aspartate aminotransferase (AST) and total bilirubin levels were measured in the serum samples obtained at sacrifice using commercially available kits (Sigma, St. Louis, Mo.)

Aspartate aminotransferase (AST) catalyses the transfer of an amino group from aspartate to oxaloacetate (OAA). The functional assay is based on the coupling of dinitrophenylhydrazine (2,4-DNP) to form a hydrazone which is blue in color [213]. Briefly, aspartate is added in excess to the serum sample to force the production of OAA [213]. Then, 2,4-DNP is added and the blue color that results is quantitated by absorbance at $\lambda=505$ nm. The absorbance is plotted on a reference curve to obtain the serum AST measurement in standard fluorometric (SF) units.

The serum bilirubin assay is based on the diazo reaction described by van den Bergh in 1918 [214]. Using this method, the bilirubin in the serum is coupled with p-diazobenzenesulfonic acid to form azobilirubin. The color of azobilirubin is pH dependant; in an alkaline environment, azobilirubin becomes blue. Measurement of the blue color by spectrophotometry is accomplished at a λ of 600 nm. A calibration curve of using known concentrations of bilirubin is prepared. Then, using the calibration curve, the bilirubin concentration can be calculated based on absorbance at 600 nm [215].

HEPATIC REGENERATION ASSAYS

Liver Weights

The weights of the resected liver specimens were measured and recorded as the "Resected Liver Mass", while the weight of liver remaining at 24 hours post-partial hepatectomy was recorded as the "Final Residual Liver Mass".

"Total Initial Liver Mass" at the time of surgery was estimated as Resected Liver Mass X (100/70). Finally, "Percent Restituted Mass" is the ratio of Final Residual Liver Mass to the calculated Total Initial Liver Mass [216].

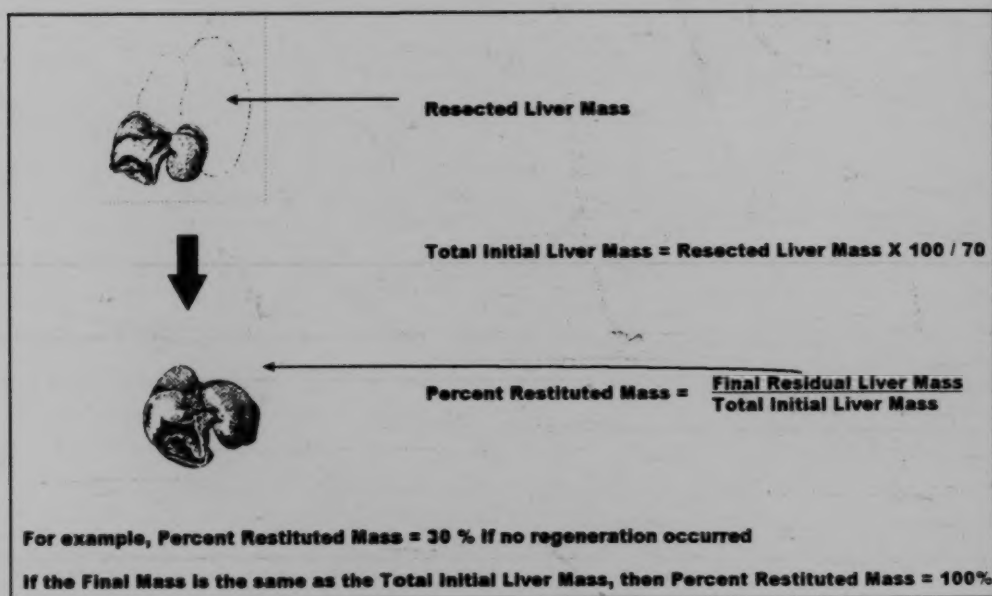


Figure V: Equations for the calculation of restituted liver mass.

Thymidine Incorporation

Thymidine incorporation assays were performed as described previously [217]. Briefly, all rats received ^3H -Thymidine 10 μCi per 200 grams body weight, by intraperitoneal injection one hour prior to sacrifice. At the time of sacrifice, remnant livers were excised and stored at -70°C until batch analyses could be performed. Approximately 0.5 grams of each liver specimen was homogenized (1:10 w/v) in a solution of EDTA 2.5 mM and aliquots were precipitated with tricarboxylic acid (TCA) to separate intracellular from free ^3H -Thymidine. The precipitates were then washed twice and solubilized overnight. ^3H -Thymidine radioactivity in the precipitates were determined using a liquid scintillation counter (Wallac LKB 1219 Rackbeta, Stockholm, Sweden).

Total DNA content was measured after reaction with DABA (3,5 diaminobenzoic acid), using a fluorometer (Sequoia-Turner Model 450 digital fluorometer).

Thymidine incorporation was expressed as counts per minute incorporated per milligram of DNA.

PCNA protein determinations

Liver homogenates were washed three times with cytosolic protein extraction buffer which contained 1% Triton X-100, 0.1% Sodium Dodecyl Sulfate (SDS), Sodium Phosphate 2 mM, and EDTA 5 mM. Protein concentrations were determined by the method of Lowry [218]. A sample of 100 μg of protein was mixed with loading buffer and denatured by boiling for 20

minutes. The denatured polypeptides were then separated by electrophoresis on a 10% polyacrilamide gel and transferred to nitrocellulose filters. The filters were incubated overnight at 4°C in a solution of PC-10 mouse monoclonal antibody specific for PCNA protein. The secondary antibody (anti- mouse IgG bound to horseradish peroxidase) was then bound over one hour. PCNA bands were visualized by autoradiography following addition of a commercially available chemiluminescence kit (ECL Western blotting analysis system, Amersham Life Science, Oakville, Ontario) and quantified using NIH densitometry software for the Macintosh computer.

QUANTITATION OF FIBROSIS

Computer Assisted Quantitation of Fibrosis

The degree of fibrosis was quantified using computer-assisted image analysis (Bioquant Automated Meg 4 program; R+M Biometrics, Inc. Nashville, TN) performed in a blinded fashion. Fibrous tissue stained deep violet by the Van Gieson method and the area of fibrosis in 5 randomly selected fields was measured and then averaged. The fibrosis index was calculated as fibrosis area per unit area of liver tissue.

Fibrosis Grading

A Ludwig's fibrosis score from 0 to III was assigned to each of the slides, in blinded fashion [47]. Grade 0 was assigned for normal liver architecture. Grade I was assigned to specimens with fibrosis increased in portal areas and sinusoidal space, but having regular liver architecture preserved. Grade II was assigned for thin fibrous septa present connecting portal areas and pseudolobules seen infrequently. Grade III was assigned for thick fibrous septa and collagen bands accompanied by pseudolobules. Grading was performed in a blinded fashion.

Gene Expression : Procollagen, MMP

Total RNA was isolated from the frozen resected liver specimens, as previously described [219]. Individual samples (~ 0.5 g) were homogenized in a solution of LiCl 3M and Urea 6M (12 mL). The pellets were then resuspended

and purified by a series of three protein extractions using phenol (1 mL) and chloroform (1 mL) followed by centrifugation at 3000 rpm for 10 minutes and resuspension of the pellet. The resultant RNA was precipitated overnight in ethanol and redissolved in ddH₂O to give an approximate concentration of 5 µg / µL. RNA concentrations were determined by measuring the optical density (OD₂₆₀) using a spectrophotometer (Bausch and Lomb Spectronic 2000).

Quantitation of RNA transcripts was performed by the Northern blotting method [220]. Samples of RNA (20 µg) from each liver were separated by electrophoresis on a 1% formaldehyde - agarose gel and blotted to nitrocellulose filters (Zetaprobe GT Genomic Membrane, Biorad, Hercules, CA).

The cDNA probes for Procollagen type I and Matrix Metalloproteinase (MMP), generously provided by A. Gahary, University of Alberta, were radiolabelled with ³²[P] dCTP using a commercially available random primer DNA labeling kit (Gibco BRL, Life Technologies, Gaithersburg, MD). The nitrocellulose filters were incubated at 42 °C with the radiolabelled probes for 24 hours, then washed three times to reduce non-specific binding. The labeled bands were visualized by autoradiography and quantified using the NIH densitometry program for the Macintosh computer.

As the levels of expression of MMP transcripts were very low, it became necessary to further purify the total RNA to exclude ribosomal RNA (which makes up 80-90% of total RNA) and obtain a preparation that consists predominantly of mRNA. This was accomplished through the use of a poly-T

labeled Sephadex bead column. Because mRNA makes up only a small fraction of total RNA, two randomly selected samples from each group were combined to provide one data point.

STATISTICAL ANALYSIS

Statistical analysis of continuous data was performed with one-way ANOVA followed by Tukey's method for multiple comparisons.

For nominal data, the chi-squared test was used. Fisher's exact test was used where one group or more had a count of less than 5.

The Kruskal-Wallace test was used for ordinal data such as fibrosis grade (0, I, II, III).

Two-tailed testing was performed in all cases, because the interventions could have potentially had beneficial as well as deleterious effects. P-values of less than 0.05 were considered significant. Results are expressed as mean $\pm$ SEM (standard error of the mean).

RESULTS

Results are expressed as $\bar{x} \pm \text{SEM}$ (standard error of the mean)

Error bars represent SEM

In all figures and tables,

CC	=	cirrhotic controls
Cipro	=	ciprofloxacin
Pentox	=	pentoxifylline
TCHR	=	traditional Chinese herbal remedy
HC	=	healthy controls

SURVIVAL

a) Induction period:

A mortality rate of 38 % (40/104), comparable to that seen in other studies, was encountered during the induction of cirrhosis. In addition, mortality and weight curves were similar to those previously reported (see Figure VI and VII) [182]. The mortality rate following each CCl_4 injection increased after the seventh dose, from 5.0 to 8.3 rats / week, corresponding to the time shrunken, fibrotic livers began to be noted in autopsies.

When the gavage treatments were started, esophageal variceal hemorrhage was noted on 12 occasions; in 4 cases, the animals exsanguinated within 24 hours. Gross ascites was noted in 13 / 104 rats (12%); two of the rats needed to be sacrificed when ascites visibly impaired respiratory efforts.

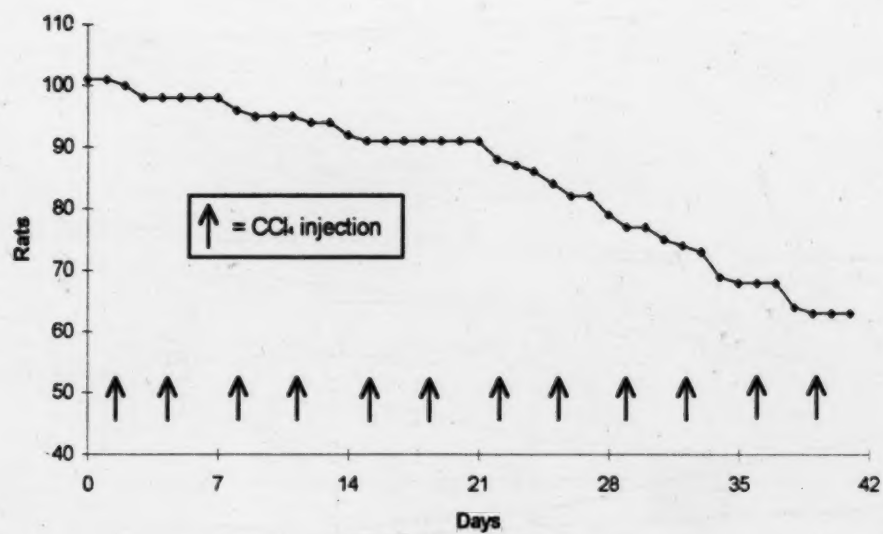


Figure VI : Rat survival curve with induction of cirrhosis.

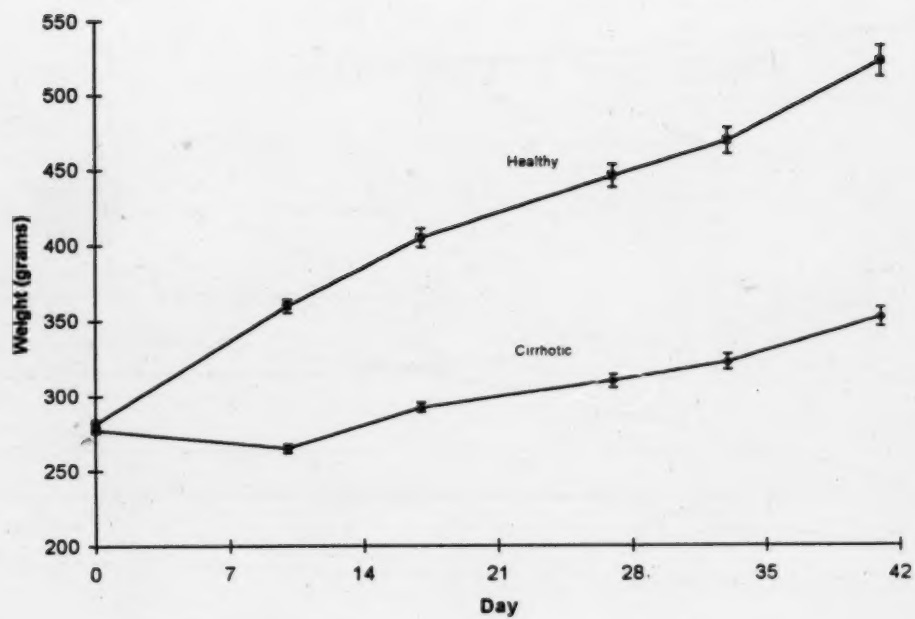


Figure VII : Rat weights during the induction of cirrhosis.

b) Treatment period

Over the four-week treatment period, 6 out of 15 (40%) of the untreated cirrhotic controls died of liver failure. Similar mortality rates occurred in the ciprofloxacin [6 / 16 (37.5%)] and TCHR [8 / 17 (35.7%)] groups. Pentoxifylline treated rats fared significantly better, with only one of 15 rats (6.6%) dying over 4 weeks of gavage treatments ($p=0.04$, compared to untreated cirrhotic controls).

Table II : Survival during the four-week treatment period.

	Survival	%	p-value*
Cirrhotic Controls	9 / 15	60.0	-
Ciprofloxacin	10 / 16	62.5	NS
Pentoxifylline	14 / 15	93.3	0.043
TCHR	9 / 17	52.9	NS
Healthy Controls	12 / 12	100	0.005

** Calculated using the Chi-squared test, compared to Cirrhotic Controls*

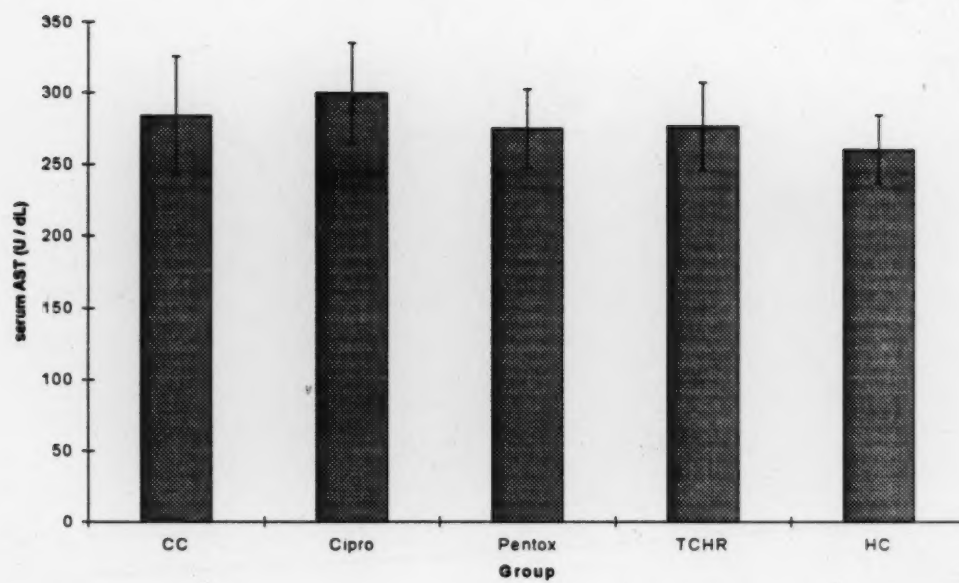
LIVER FUNCTION

Serum AST (Figure VIII)

As shown in Figure VIII, serum AST levels were similar in all five groups at 24 hours postoperatively. That AST levels were similar in healthy and untreated cirrhotic controls suggests that the inflammation secondary to CCI_4 administration had resolved.

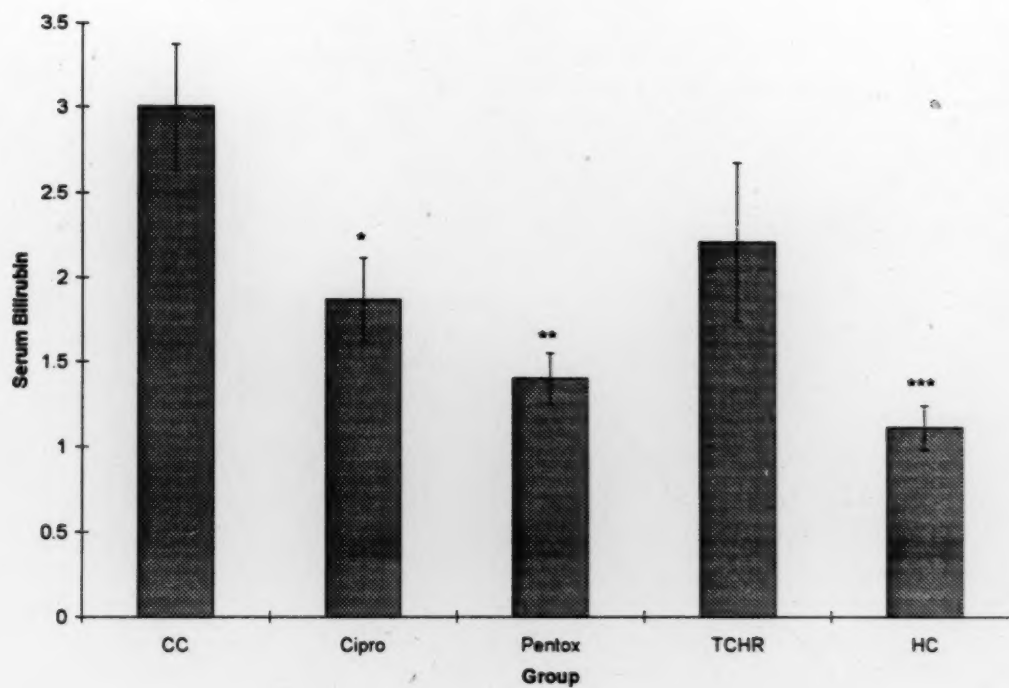
Serum Bilirubin (Figure IX)

Serum bilirubin levels were significantly higher in the untreated cirrhotic rats (3.00 ± 0.37 mg / dL, $n=6$), when compared to untreated healthy controls (1.10 ± 0.15 mg / dL, $n=12$). When compared to the untreated cirrhotic controls, the ciprofloxacin treated group (1.87 ± 0.25 mg / dL, $n=9$, $p<0.05$) and the pentoxifylline treated group (1.40 ± 0.15 mg / dL, $n=11$, $p<0.01$) had significantly lower serum bilirubin levels, suggesting improved hepatic function. Serum bilirubin levels in the TCHR treated group (2.20 ± 0.45 mg / dL, $n=9$) were not significantly improved over those of the untreated cirrhotic controls.



No significant differences.

Figure VIII : Serum AST 24 hours post partial hepatectomy (N=6-11 rats/group)



* $p < 0.05$
** $p < 0.01$ compared to CC group
*** $p < 0.005$
(using ANOVA, followed by Tukey's test for multiple comparisons)

Figure IX : Serum bilirubin 24 hours post-partial hepatectomy (N=6-11 rats/group)

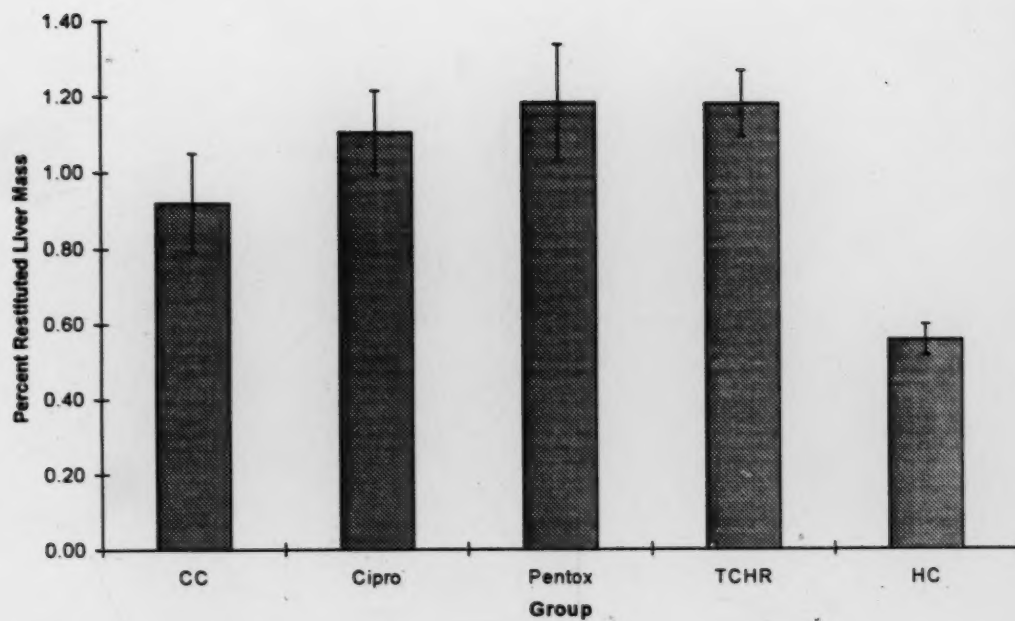
HEPATIC REGENERATION

Liver Weights (Figure X)

In the four cirrhotic groups, restituted liver mass was similar to or appeared to exceed the estimated initial mass of the liver. In healthy controls, 50% of the estimated initial mass was present at 24 hours post partial hepatectomy.

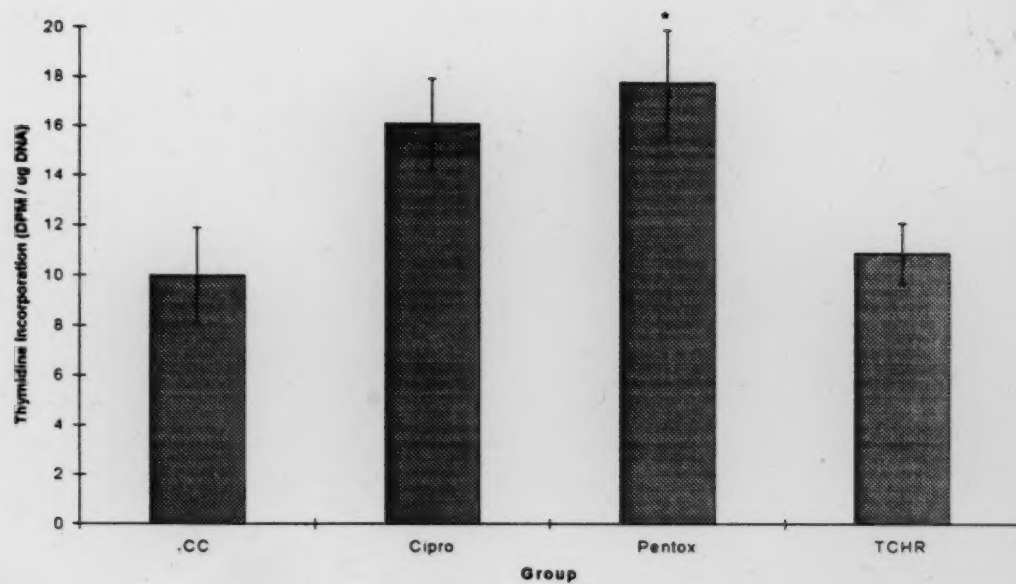
$^3\text{[H]}$ -Thymidine Incorporation (Figure XI and XII)

Rats treated with pentoxifylline had a higher rate of $^3\text{[H]}$ -Thymidine incorporation when compared to untreated cirrhotic controls (17.8 ± 2.2 vs. 9.9 ± 1.9 DPM / μg DNA, $p=0.05$). The other two cirrhotic groups had $^3\text{[H]}$ -Thymidine incorporations similar to that observed for the untreated cirrhotic control group (ciprofloxacin 16.1 ± 1.8 DPM / μg DNA ($p=0.09$), TCHR 10.9 ± 1.2 DPM / μg DNA ($p>0.2$)). Overall, as shown in Figure XII, rates of $^3\text{[H]}$ -Thymidine incorporation remained poor for all four cirrhotic groups when compared to the healthy control group (92.2 ± 17.5 DPM / μg DNA).



* $p < 0.05$ using ANOVA followed by Tukey's test for multiple comparisons.

Figure X : Percent Restituted Liver Mass 24 hours post-partial hepatectomy (N=6-12 rats/group)



* $p = 0.05$ using ANOVA followed by Tukey's test for multiple comparisons.

Figure XI : Thymidine Incorporation 24 hours post partial hepatectomy (N=6-11 rats/group)

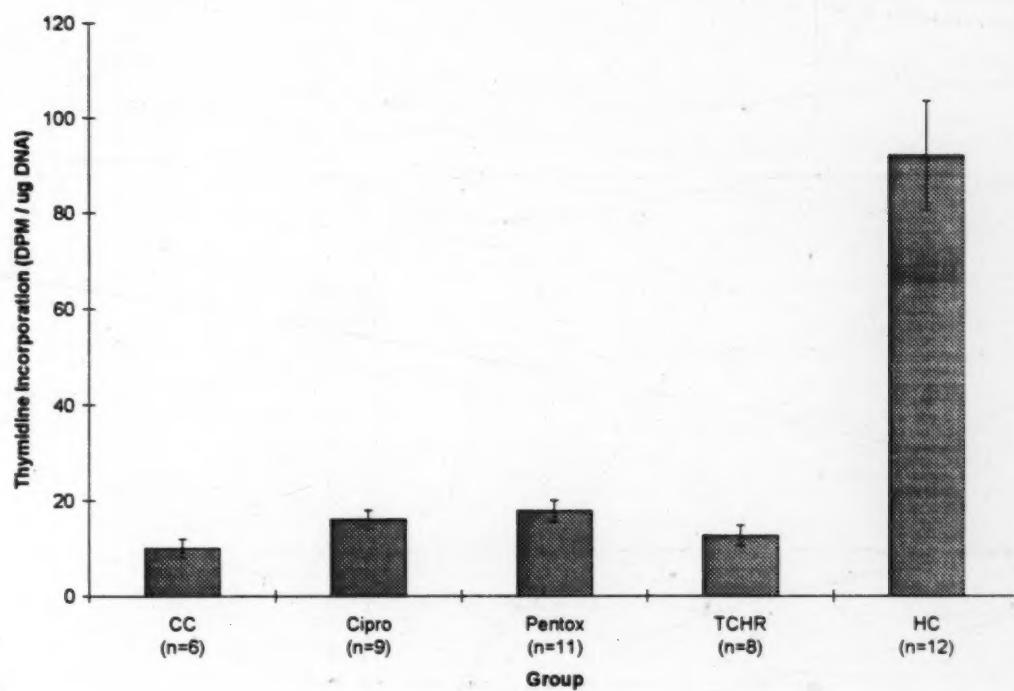
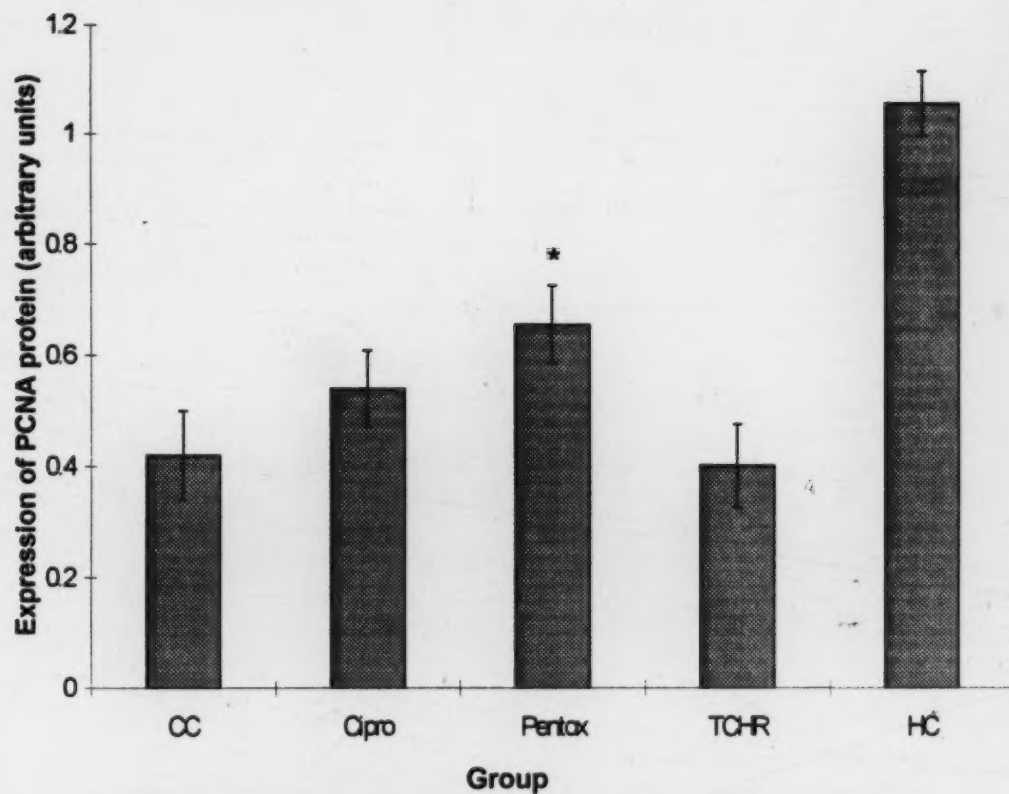


Figure XII: Thymidine incorporation, including healthy controls.

PCNA Protein Assays

The results of PCNA protein determinations were in keeping with the ^3H -Thymidine incorporation findings. The correlation between ^3H -Thymidine incorporation and PCNA protein was strong ($R = 0.74$, $p < 0.0001$).

Once again, when compared to the healthy controls, PCNA protein levels in the four cirrhotic groups were markedly impaired. The ciprofloxacin and TCHR groups had levels similar to those of the untreated cirrhotic control group (0.54 ± 0.07 u and 0.40 ± 0.08 u respectively). While there was a trend toward greater PCNA expression when the pentoxifylline group (0.66 ± 0.06 u) was compared to the untreated cirrhotic control group, (0.42 ± 0.08 u) the difference was not statistically significant ($p=0.23$).



* $p = 0.23$, compared to the CC group.

Figure XIII : PCNA protein expression 24 hours post-partial hepatectomy (N=6-12 rats/group). A representative autoradiograph of PCNA expression is shown below each group.

FIBROSIS

Computer Assisted Quantitation of Fibrosis (Figure XV and XV)

A representative section for each of the four cirrhotic groups is shown in Figure XIV (van Gieson stained, 50X).

Healthy controls had a mean fibrosis index of 0.9 ± 0.1 %. Fibrosis appeared slightly improved in each of the treated cirrhotic groups compared to the untreated cirrhotic control group (8.1 ± 0.7 %). However, only the ciprofloxacin treated group (5.5 ± 0.6 %, $p < 0.05$) and the pentoxifylline treated group (4.6 ± 0.2 %, $p < 0.005$) reached statistical significance. Although the TCHR treated group tended toward improvement, the large variance contributed to the results not reaching statistical significance (6.6 ± 1.1 %).

Fibrosis Grade (Table III)

The results of fibrosis scoring from 0 - III are shown in Table III. The only statistically significant differences were those between the healthy controls and each of the cirrhotic groups.

When data are grouped into severe fibrosis (grade III) versus non-severe grades (0-II), as in Table IV, significant improvements are seen for the pentoxifylline treated ($p = 0.001$) and ciprofloxacin treated groups ($p = 0.03$), compared to the untreated cirrhotic controls.

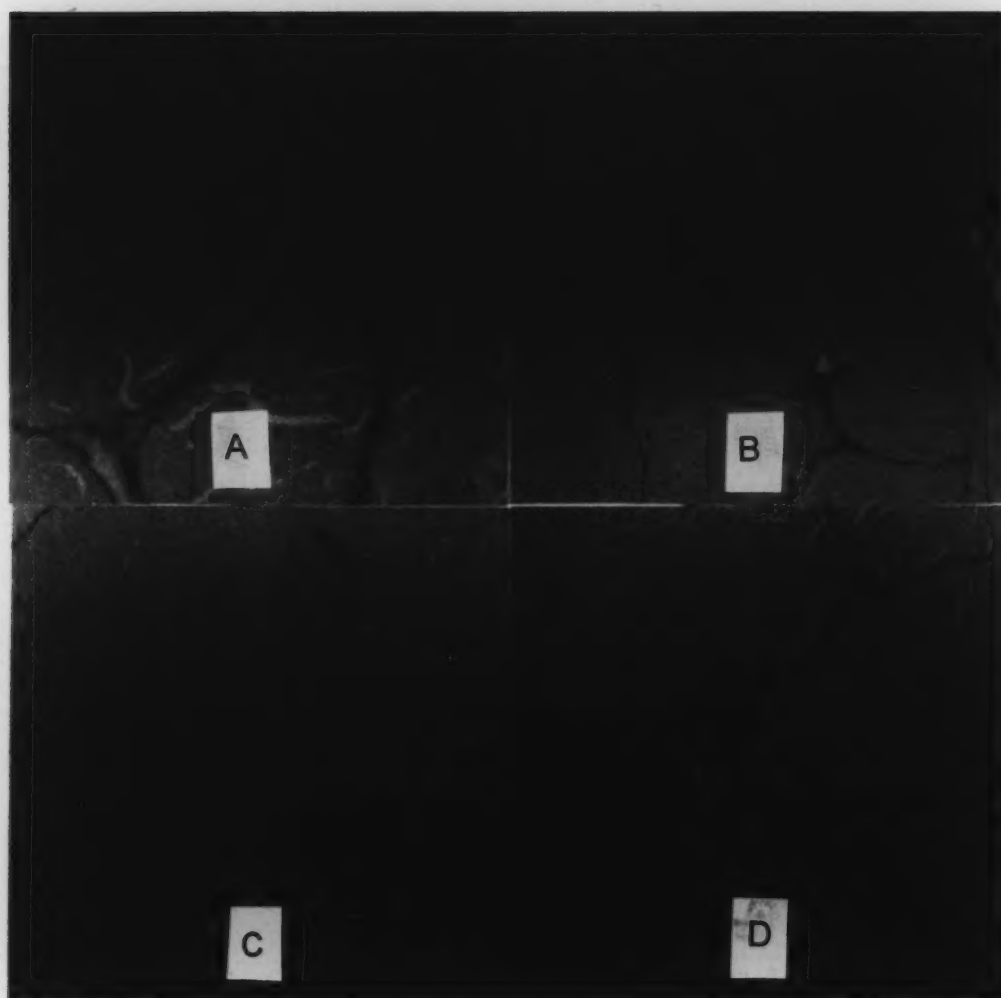
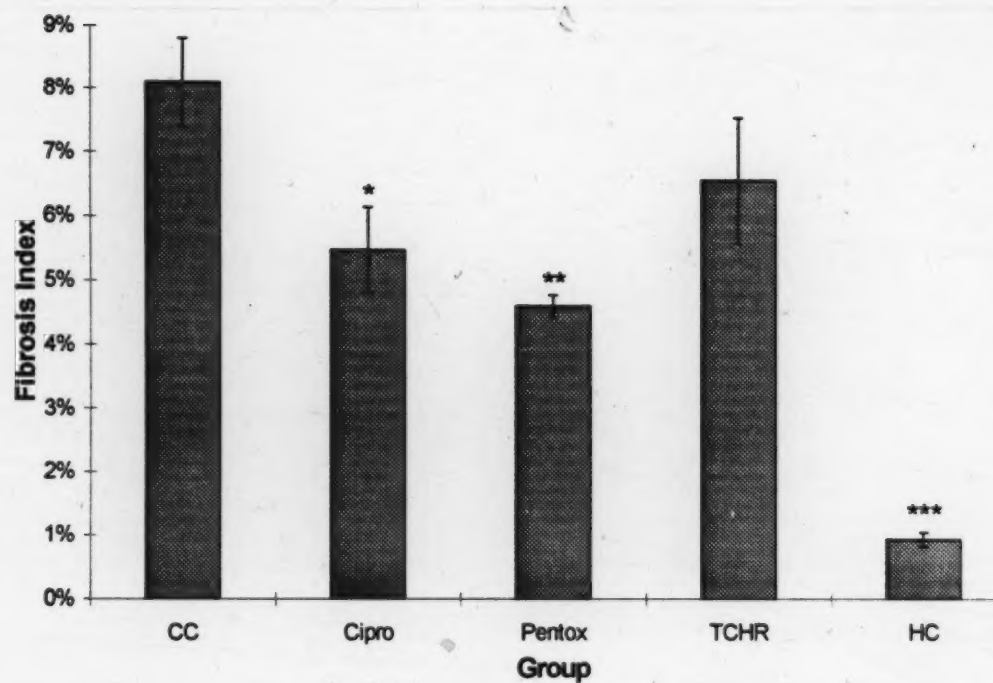


*Figure XIV : Representative sections from each of the four cirrhotic groups (van Gieson, 50X)
(A) cirrhotic controls, (B) ciprofloxacin, (C) pentoxifylline, (D) TCHR.*



* $p < 0.05$

** $p < 0.005$

*** $p < 0.0001$

compared to CC group

Figure XV : Fibrosis index

Table III : Fibrosis grade for the five groups.

Group	0	I	II	III
Cirrhotic Controls	0	1	1	7
Cipro	0	3	4	2
Pentox	0	3	9	1
TCHR	0	2	4	5
Healthy Controls	11	1	0	0

No significant differences between groups.

Table IV : Severe versus mild to moderate fibrosis.

Group	0-II	III
Cirrhotic Controls	2	7
Cipro	7	2 [*]
Pentox	12	1 ^{**}
TCHR	6	5
Healthy Controls	12	0 ^{***}

^{*} $p < 0.05$

^{**} $p = 0.001$

^{***} $p < 0.001$

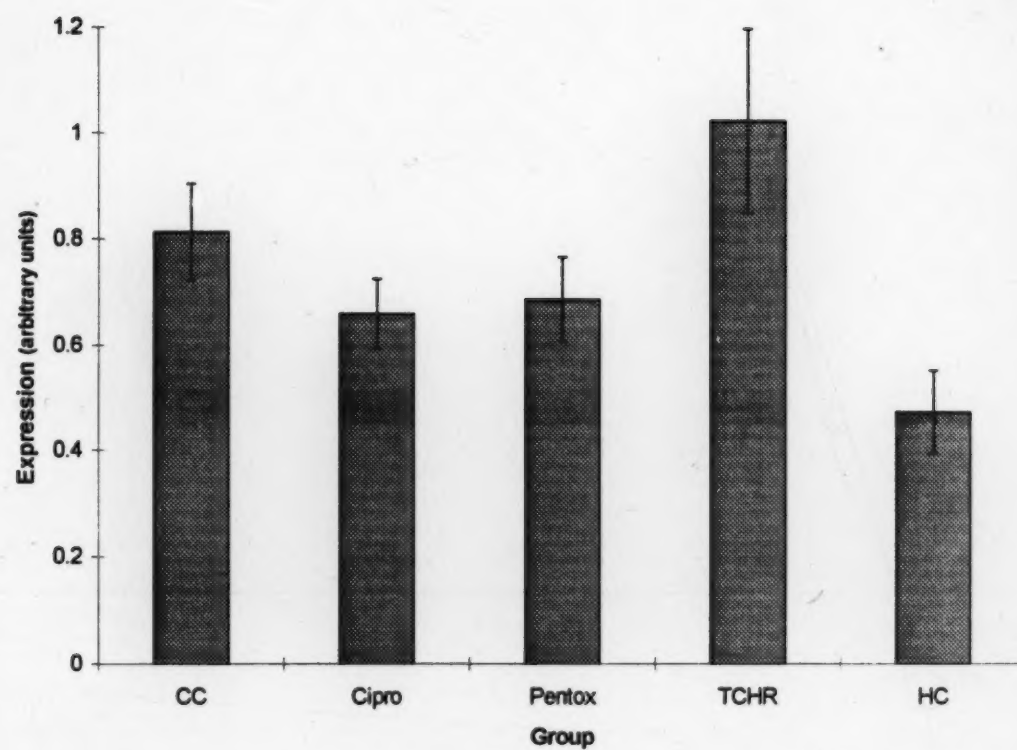
compared to CC group, using Fisher's exact test.

Procollagen type I mRNA expression

Procollagen type I mRNA expression is shown in Figure XVI. Expression was similar for the four cirrhotic groups. The only significant differences were between the healthy controls, and the untreated cirrhotic control group and the TCHR treated group (0.47 ± 0.08 u vs. 0.81 ± 0.09 u ($p < 0.05$) and 1.02 ± 0.17 u ($p < 0.01$), respectively).

Matrix Metalloproteinase mRNA expression

As mentioned earlier, it was necessary to use the mRNA fraction of total RNA to probe for levels of TGF- β and MMP, and samples needed to be combined to yield sufficient mRNA. Unfortunately, some of the paired samples contained degraded RNA which resulted in uninterpretable data. However, in lanes where RNA was intact, results did not suggest differences between the five groups in terms of MMP expression (data not shown).



* $p < 0.05$ compared to the CC and TCHR groups using ANOVA, followed by Tukey's test.

Figure XVI : Procollagen type I, alpha 1 chain mRNA expression.

DISCUSSION

SUMMARY

In summary, of the preoperative interventions tested, pentoxifylline emerged as the agent with the most promise for improving the outcome of partial hepatectomy in the setting of cirrhosis. Specifically, pentoxifylline administered for four weeks improved survival, postoperative function, and fibrosis, compared to untreated cirrhotic controls. Regeneration was improved but remained impaired when compared to healthy controls.

Ciprofloxacin, despite its previously documented effect on enhancing hepatic regeneration following short-term use, did not significantly improve regeneration in these cirrhotic rats when given for four weeks. Benefits were, however, seen in terms of decreased fibrosis and improved levels of postoperative serum bilirubin, suggesting that non-regenerative effects may be operative.

The formulation of traditional Chinese herbal remedy (TCHR) used in this study failed to show significant benefits in terms of any of the outcomes examined.

CIPROFLOXACIN

Many studies have been performed examining the beneficial effects of ciprofloxacin on the liver due to its GABA receptor blocking properties [161,162,163]. These studies have documented an enhancement of regenerative activity as well as protection against the inhibition of regeneration by ethanol [166]. A recent, unpublished study suggests a protective effect (improved survival and regenerative activity) in a galactosamine-induced rat model of fulminant hepatic failure [167].

In one study reported to date on the use of ciprofloxacin as a treatment for cirrhosis, Zhang and colleagues administered ciprofloxacin 50 mg / kg twice daily by gavage to cirrhotic rats for four weeks [77]. The result was a decrease in inflammation, as measured by ALT, and a decrease in fibrosis, as measured by tissue proline and hydroxyproline levels. Regenerative potential, however, was not measured.

In our study, serum ALT values were similar to healthy controls suggesting that the toxic effects of CCl₄ administration had likely resolved. In addition, we observed a significant improvement in total serum bilirubin.

Although we assessed fibrosis by different means than those used by Zhang et al, we also found that collagen content (as quantified by the fibrosis index) was improved when compared to untreated cirrhotic controls.

Our results suggest that ciprofloxacin may decrease fibrosis. Whether this is a primary effect (decreased fibrogenesis or increased fibrinolysis), or simply

secondary to improved regeneration that occurred during the treatment phase of the study remains to be determined. Preliminary Procollagen type I and MMP results tend to favor the latter explanation.

While previous studies documented a beneficial effect of ciprofloxacin on regeneration, the results in this study showed that regeneration after partial hepatectomy was unchanged following four weeks of treatment with ciprofloxacin. It is important to recall that the purpose of this study was to examine the usefulness of these interventions in the preoperative preparation prior to liver resection. The treatments were stopped the day prior to surgery—therefore, negligible serum concentrations of the drug would be expected to remain at the time of surgery. Little is known about the duration of the effect of ciprofloxacin on the GABA receptor.

Other possibilities for the lack of significant improvement in regeneration include the daily rather than twice daily treatment schedule employed during the first half of the four week treatment period. Alternatively, it is conceivable that resistance to the long-term administration of ciprofloxacin occurs because of either increased metabolism of the drug or GABA receptor upregulation, for example. Different scheduling and doses of ciprofloxacin may be needed to reproduce the promising effects described in earlier studies. Further studies are therefore required to determine the effects of long-term ciprofloxacin administration on the cirrhotic liver.

PENTOXIFYLLINE

Peterson and colleagues have investigated the use of this methylxanthine on liver fibrosis for the last ten years, using both *in vitro* and *in vivo* models [71]. The *in vivo* studies were largely carried out on the yellow phosphorus induced cirrhotic pig model [20]. The effects of pentoxifylline in a rat model have yet to be reported.

In their *in vivo* studies, Peterson and associates documented the effects of administering pentoxifylline 16 mg / kg daily during the 12 week period of induction of cirrhosis using yellow phosphorus. They found that pentoxifylline reduced the levels of γ -GGT and alkaline phosphatase and decreased collagen content, while improving the histological appearance [20]. These results were in agreement with their *in vitro* results suggesting that pentoxifylline decreases PDGF-induced fibroblast proliferation, possibly by acting as a competitor of the PDGF receptor [71].

Our findings are consistent with those reported by Peterson, and in addition, we have studied effects on survival, liver function, and hepatic regeneration. In our study, survival during the treatment phase was improved as a result of pentoxifylline treatment. AST values remained unaltered, but postoperative serum bilirubin was markedly improved. A significant improvement was also observed in ^3H -Thymidine incorporation. However, we performed PCNA protein quantitation by Western blotting, as another measure of regeneration which does not depend upon the uptake of a tracer and were

unable to find significant differences between the groups. These data suggest either that there is no improvement in regeneration in the pentoxifylline treated group when compared to the untreated cirrhotic controls, or that the difference is very small. In addition, pentoxifylline significantly improved the fibrosis index as assessed by computer assisted quantitation.

The most marked effect appeared to be on postoperative function at 24 hours; total serum bilirubin levels were similar to those found in the non-cirrhotic group, a marked improvement over the untreated cirrhotic control group. In the absence of differences in inflammation, there are two possible explanations for the improvement in survival and function: increased regeneration or decreased fibrosis. The improvement in regeneration was limited, and it is possible that this avoided the deterioration in liver function that is associated with rapid regenerative activity. More likely however, the improved function resulted from decreased fibrosis. The lack of preoperative serum bilirubin levels does not allow us to draw any strong conclusions on the mechanism whereby function was improved. In other words, preoperative function may have been much better for the pentoxifylline treated rats, allowing them to have lower serum total bilirubin postoperatively as well.

It has been hypothesized that collagen deposition, particularly in the area of the space of Disse may be responsible for both the impaired function and regeneration seen in cirrhosis [14]. The space of Disse is normally filled with loose connective tissue which allows the uptake of substances from the

bloodstream into hepatocytes and the free exchange of molecules from the hepatocyte to the bloodstream. Thus, an improvement in fibrosis could have the effect of improving hepatocyte function, presumably to a greater extent than improving regenerative activity.

Improved regeneration in the pentoxifylline group may be the result of a more favorable extracellular matrix which allows for more efficient cell-to-cell communication and less restriction of tissue growth.

An alternative explanation for the increased regeneration is that the hemodynamic effects of pentoxifylline were such that ^3H -Thymidine delivery to hepatocytes was enhanced. We attempted to rule out this possibility by performing PCNA protein assays, which do not depend upon the uptake of a tracer. While a strong correlation existed between ^3H -Thymidine uptake and PCNA protein expression, improved ^3H -Thymidine uptake may account for an improvement in regeneration as measured by ^3H -Thymidine incorporation, but not by PCNA protein assay.

Another parameter which was markedly improved in the pentoxifylline treated group was the degree of fibrosis, as measured by computer assisted quantitation, as well as the fibrosis scores. These findings are similar to those reported by Peterson in the yellow-phosphorus induced cirrhosis model in the pig [20]. The mechanism whereby pentoxifylline decreases fibrogenesis is likely to be by the inhibition of PDGF at the receptor or post-receptor stage.

Recent work by Wanless and associates has suggested a new mechanism for hepatic fibrosis [221]. Their group initially revisited the uncommon disease process involved in cardiac cirrhosis, in an attempt to elucidate the mechanism whereby the fibrotic changes occur in this disease. A striking correlation was seen between the regions having thrombosis in the small and medium hepatic venules and regions containing hepatic fibrosis. The process is termed "parenchymal extinction". A subsequent study suggested a similar mechanism may be involved in the pathogenesis of HCV cirrhosis [222].

A study in our lab examined retrospectively the effect of a hypocoagulable state, such as that seen with hemophilia, on the progression of HCV cirrhosis [223]. It was hypothesized that these patients would be less prone to thrombotic events, such that the progression of cirrhosis would be less rapid. Patients with hemophilia and chronic HCV infection from three centers were matched with non-hemophilic patients also with chronic HCV. The degree of fibrosis in the hemophilic group was significantly less, even when compared to matched HCV-infected non-hemophilic controls with half the duration of their disease.

These studies suggest that thrombosis in the small hepatic vessels appears to be involved in the pathogenesis of fibrosis. The prevention of thrombosis is another mechanism whereby pentoxifylline could have improved the fibrotic state of the liver.

TRADITIONAL CHINESE HERBAL REMEDY

The effects of a formulation of TCHR in a rat model of cirrhosis were first reported in the study by Zhang discussed above [77]. The results included a decrease in inflammation as measured by ALT, as well as a marked decrease in fibrosis. Indeed, the architectural distortion seen in cirrhosis appeared to be almost reversed to normal.

While this formulation of TCHR showed impressive effects in the study by Zhang et al., the same formulation failed to show any significant benefit in our study. Upon closer inspection, this study differed from Zhang's in the following ways:

1. The species of rat used: Wistar rats were used in the previous study, while Sprague-Dawley rats were used in this study. While numerous differences between the two species likely exist, a size discrepancy was clearly apparent. Ten weeks after the start of the Zhang study, the healthy control Wistar rats weighed approximately 300 grams, whereas some of the rats in our study weighed over 600 grams.
2. The cirrhosis model: In addition to CCl_4 injections and ethanol in the drinking water, Zhang's model included a special choline reduced diet to enhance liver damage. The effects of the choline-reduced diet are complex and experience with this model is limited. Effects of a choline reduced diet on the liver include hepatocyte cell death, fatty deposition, and induction of hepatomas in certain rat models [224,225]. Cell

proliferation occurs in response to ongoing cell death; this proliferation has been shown to persist for several months following the return of the animals to a normal choline diet [226]. One group observed that in a model of refeeding following nutritional deprivation, the liver became primed for regeneration [227]. A similar mechanism may have been at work in the rats fed a choline reduced diet and then returned to regular diets.

3. The herbal medicine ingredients: Some of the ingredients for the herbal remedy were obtained from sources different from those used in the study by Zhang. One of the problems with the use of herbal medicines is the lack of uniformity or measurable standards. Hence, it is difficult to determine a standard, reproducible dosage. Different plant species, soil, or harvesting techniques, for example, could significantly alter the remedies' therapeutic properties [228]. For instance, ginseng grown in California may contain a low or negligible amount of a key substance such as ginsenosides, compared to levels obtained in Korean ginseng.

REGENERATION

Liver regeneration was not improved to a large extent by any of the interventions in the cirrhotic groups. While it is known that severely fibrotic livers have poor regenerative capacities, the precise relationships between the degree of fibrosis, function, and regenerative capacity remain unclear. It is likely that the three are closely related. Adequate postoperative function depends to

some degree on the regenerative response, while the ability for the liver to function and regenerate normally likely depends on the degree of fibrosis. On the other hand, as suggested by Ito and associates, excessive regeneration may contribute to impaired function [86].

A number of possible explanations have been proposed for the impaired regeneration that is seen in cirrhotic livers, including increased levels of circulating inhibitors [42], abnormal extracellular matrix, contributing to abnormal cell-to-cell communication, and hepatocyte abnormalities [14]. All of these factors probably contribute in some way to the problem.

Collagen deposition is central to the distortion of hepatic parenchymal architecture, and excessive collagen deposition, particularly in the space of Disse has been proposed to interfere with normal hepatocyte function and regeneration. Thus, an important part of rectifying the impaired function and regeneration seen in cirrhotic liver is likely to require some correction of the collagen deposition. The severity of the architectural distortion seen in cirrhotic liver suggests a need for a prolonged period of therapy, such as the four week period examined in this study.

In this study, despite improvements in postoperative function, histological appearance, and degree of fibrosis, for some groups, regeneration remained less than predicted based on the results of previous studies and in comparison to healthy controls. Several explanations are possible:

1. The proportion of liver resected may have been less than 70%. Two studies have shown that the extent of resection greatly impacts on the size of the regenerative response [186,189]. Regeneration, as measured by ^3H -Thymidine incorporation, for resections of 34 and 43 percent, is but a fraction of the regeneration that occurs following 68% resections. Similar results were obtained in a study performed by the author [188]. Following 55% and with more extensive 90% resections, we noted that regeneration was significantly decreased when compared to 70% resections. Thus, the extent of resection is an important determinant of regenerative activity. In the present study, extents of resection were difficult to quantitate. At the time of surgery, the caudate lobe, which is usually very small and posterior was noted to protrude anteriorly. Similar anatomical distortions have been reported in cirrhotic livers in humans [229]. In three rats that died intraoperatively, the resected portion weighed 6.7 ± 0.8 grams, while the remaining lobes weighed 6.2 ± 1.3 grams. This would suggest that the mass resected in these animals was closer to 50% than 70%. The reason for the distortion in the size of the liver lobes may be related to the severely diseased state of the livers, erratic delivery of the CCl_4 , and / or differences in lobular blood flow and drainage. Regardless of the cause, if in fact only 50% of liver mass was resected in the cirrhotic groups, it would seem inappropriate to compare these results to healthy

controls which had undergone 70% resections. When the rate of ^3H -Thymidine incorporation observed in the cirrhotic rats is compared to rates seen following 55% resection in noncirrhotic Sprague Dawley rats in another study [188], as shown in Figure XV, (32.1 ± 3.4 CPM / μg DNA at 24 hours, $n=3$), the statistically significant improvement seen with pentoxifylline treated rats becomes more even more apparent.

2. The timing and size of the peak of DNA synthesis in the setting of liver disease may be altered from that documented in healthy livers. Sampling DNA synthesis 24 hours post resection may not have represented peak regenerative activity. The addition of a second time point at 48 or 72 hours might have allowed the detection of a late peak. Nonetheless, it is clear that all four cirrhotic groups showed an impaired rate of DNA synthesis at 24 hours post partial hepatectomy when compared to healthy controls.
3. The treatments were simply not effective in improving regeneration in this setting.

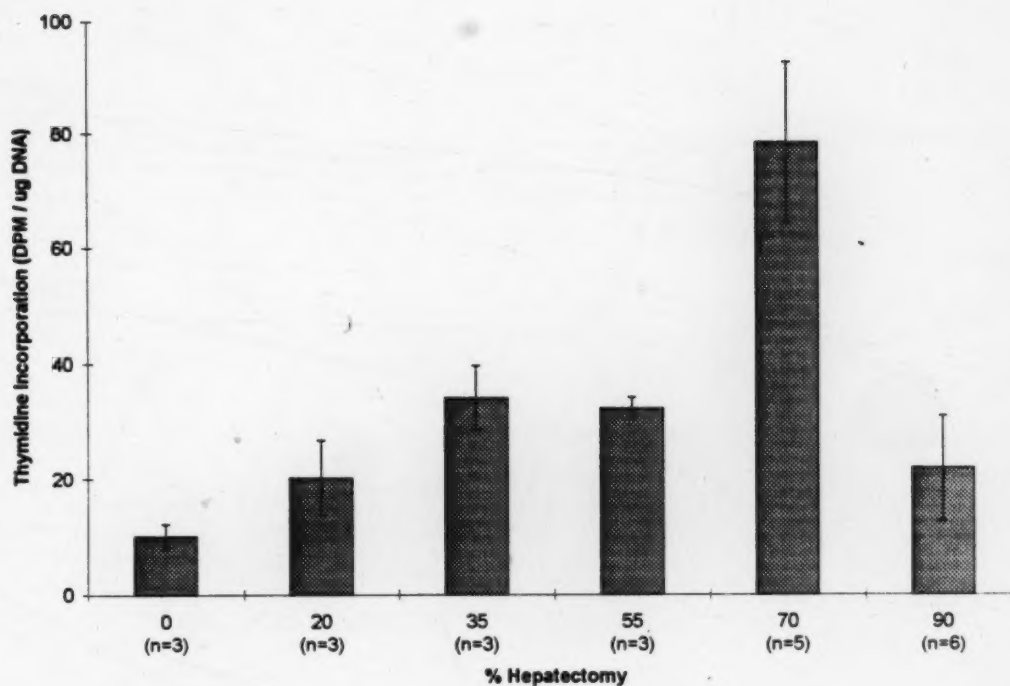


Figure XVII : Thymidine incorporation as a function of percent resected liver mass from another study. Note that the regenerative response is greatest following 70% hepatectomy, and that slightly lesser or greater resections are associated with markedly diminished regeneration. $^3\text{[H]}$ -Thymidine incorporation at 35 %, 55%, 70%, and 90% differ significantly from that following 0% resection. In addition, $^3\text{[H]}$ -Thymidine incorporation following 70% hepatectomy was much higher than the values for 55% and 90% resections ($p < 0.001$ and $p < 0.005$, respectively).

OTHER POTENTIAL PROBLEMS

One problem encountered in studies of cirrhosis is the heterogeneity of the disease process. The histological appearance of liver tissue in various regions of the cirrhotic liver may differ significantly, and this is an ongoing problem with liver biopsy interpretation. In addition, the extent of cirrhosis produced by the various induction protocols can be quite variable between rats. Thus the rats were randomized by computer to their various groups only after the six week induction of cirrhosis period was completed. The inhomogeneous nature of cirrhosis emphasizes the need for large group sizes to permit the drawing of meaningful conclusions.

The assessment of liver regeneration continues to be debated. The measurement of ^3H -Thymidine incorporation, the most commonly used method, has the disadvantage that a radiolabelled tracer is used and that parenchymal as well as nonparenchymal cells take up the tracer. On the other hand, nonparenchymal cell proliferation at 24 hours post partial hepatectomy is thought to be minimal [27]. The effect of cirrhosis on metabolism and uptake of the tracer, both of which could lead to deceiving results has not yet been documented.

To minimize these concerns, we utilized a second, intrinsic measure of regeneration, the PCNA protein assay, which is non-radioactive and not dependant on hepatic blood flow or hepatocyte uptake mechanisms. In our study the results of ^3H -Thymidine incorporation showed a strong correlation

with PCNA protein levels ($R = 0.74$, $p < 0.001$). The net effect of abnormal uptake and metabolism of ^3H -Thymidine would thus appear to be minimal. Nonetheless, until it is known precisely how cirrhosis affects ^3H -Thymidine uptake measurements, most authorities would continue to recommend the use of two measures of regeneration [200].

The lack of any differences between the groups in terms of procollagen I and MMP mRNA while differences in collagen deposition are present may be related to the fact that the rats did not obtain medication for 48 hours prior to the samples being collected. Serum levels of ciprofloxacin and pentoxifylline would have been very low at the time the samples were obtained. Whatever action the drugs were exerting may have worn off. On the other hand, the reduction in fibrosis may have occurred independently of Procollagen I and MMP, and may have resulted from increased regeneration throughout the treatment period.

CLINICAL RELEVANCE

The usefulness of any conclusions based on the CCl_4 model of cirrhosis depends upon how well the model represents human cirrhosis. Once again, the CCl_4 model of cirrhosis has been evaluated on several occasions over the last fifty years and is thought to represent a valid model of human cirrhosis. The histological and biochemical features of the model appear similar to that observed in human liver disease [184,185].

The treatments examined in this study, particularly pentoxifylline and ciprofloxacin, are already commonly used in the clinical setting, and both are available in oral and intravenous forms.

Pentoxifylline (Trental ®) has been used clinically for many years in the treatment of peripheral vascular disease. There exists a therapeutic window of serum concentrations where the drug is effective, whereas higher doses produce toxic side effects [171]. The side effects most commonly seen in humans include gastrointestinal disturbances such as nausea and diarrhea. Other more serious side effects, such as dysrhythmias and seizures, occur much more rarely [171]. The drug is metabolized by the liver, and as a result, elimination half-time is prolonged in patients with cirrhosis. The use of a lower dosage and careful monitoring would be necessary in order to safely administer pentoxifylline to cirrhotic patients.

Ciprofloxacin is commonly used as a broad spectrum antibiotic in the treatment of urinary tract infections or more serious gram negative infections. One of its main advantages is that following oral administration it is extremely well absorbed, and thus has a high bioavailability [154]. Its side effects include gastrointestinal upset, rashes, allergies, and rarely, seizures, presumably related to its GABA receptor blocking properties [160]. One potential problem with the long-term use of antibiotics is that colonic flora can be altered, increasing the risk of pseudomembranous colitis. Nonetheless, ciprofloxacin could be used quite safely in the setting of cirrhosis.

One concern which was raised in the planning of the project was the feasibility of a four week treatment period prior to hepatic resection for malignancy. As discussed earlier, HCC is the most common indication worldwide for hepatic resection, and its treatment is often complicated by coexisting cirrhosis. While most patients present at an incurable stage, some present with curable disease. In addition, it has been documented that up to 85% of HCC's are unifocal and that doubling times may be as long as 16 months [4]. Ideally, treatment could be instituted in the immediate peri-operative period. However, improvement in post operative outcome is more likely to require weeks of preparation to at least partly overcome the abnormal cell-to-cell communication and distorted architecture in the cirrhotic liver. A two to four week period of preoperative treatments, for example, may allow large gains in the aggressiveness with which resections can be carried out, while only slightly increasing the opportunity for growth of the malignancy. Certainly, a two to four week time period between the time of diagnosis and the time of surgery is not uncommon. That these interventions appeared to have their greatest effect on decreasing collagen content rather than increasing regeneration renders stimulation of tumor growth kinetics less likely.

Based on a recent study by Ito and associates [86], it has been suggested that hepatocytes undergoing regeneration enter a state where synthetic and metabolic functions are downregulated. Thus, an excessive regenerative response could actually contribute to impairment in liver function and even

death. As shown in Figure XVIII, when we consider that a patient with cirrhosis has perhaps 30% of the functioning hepatocytes of a healthy liver, then giving up 30% of liver function so that regeneration can take place has marked consequences on an already borderline liver. If the regenerative response is increased such that one-third of hepatocytes enter into regeneration, then function is severely compromised. This situation is somewhat analogous to 'flogging a dead horse'. Thus, it is likely that a patient with cirrhosis would benefit more from interventions designed to prepare the liver to tolerate surgery than from perioperative interventions designed to produce an aggressive regenerative response immediately following surgery.

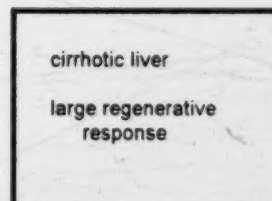
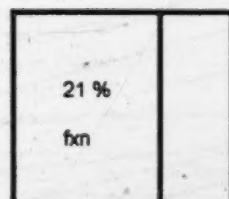
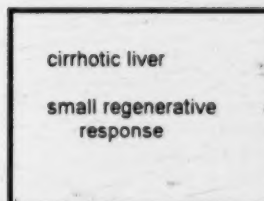
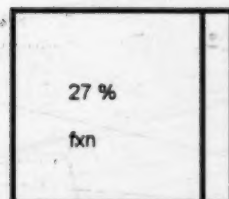
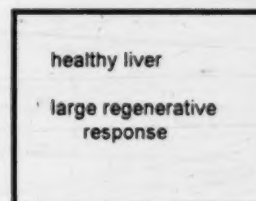
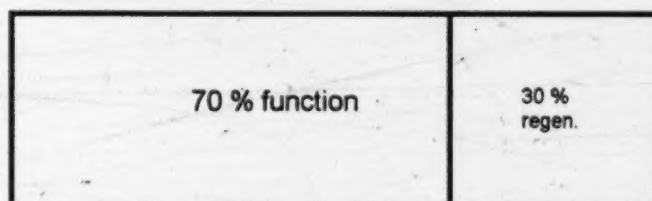
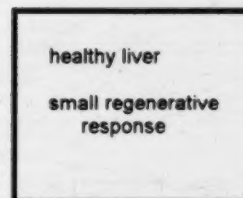
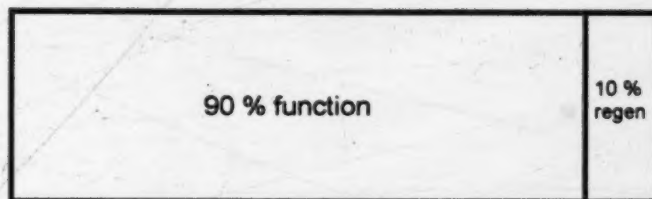


Figure XVIII : Diagram illustrating the effect of increasing regeneration on liver function in the setting of cirrhosis.

CONCLUSIONS

Of the interventions tested, pentoxifylline appears to be the most promising in improving the outcome following resection of cirrhotic liver. Pentoxifylline treatment for four weeks prior to resection significantly improved preoperative survival, postoperative function, and fibrosis in this rat model of cirrhosis. The effect on regenerative potential is less clear. The usefulness of pentoxifylline in improving postoperative survival should be confirmed with a survival study.

Ciprofloxacin, despite improving fibrosis and postoperative function, did not improve regeneration as might have been expected.

Finally, despite the promising results shown in Zhang's study [77], TCHR failed to show any significant benefit over saline gavage in our study. Such differences suggest that the ingredients used may have been different in composition than those used in Zhang's study and a reappraisal of TCHR using the original ingredients may be warranted.

TOPICS FOR FUTURE STUDY

1. Pentoxifylline appeared to be the most beneficial treatment in this study in terms of preoperative survival and postoperative function. The optimal dose and timing of pentoxifylline administration should be determined in future studies. Also, a survival study should be done to determine if pentoxifylline improves postoperative survival in the early as well as late postoperative period.
2. The mechanism whereby pentoxifylline improves the outcome in cirrhotic livers should be sought.
3. As noted above, further study is needed to define the optimal dose, scheduling, and long-term effects of ciprofloxacin on the liver.
4. Because the TCHR showed such promising results in Zhang's study, it would be worthwhile to repeat these studies using ingredients obtained from China.

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